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Endowment fund of the International Association for Dental Research (as of March, 1956): $1,802.34. There have been no expenditures from this fund.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
36th General Meeting
Hotel Statler, Detroit, Mich.
March 20-22, 1958

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EDITOR'S VIEWPOINT

An innovation is presented in this issue of the JOURNAL—the *preprinted* abstracts of the papers to be presented at the General Meeting of the International Association for Dental Research in Detroit on March 20, 21, 22, and 23, 1958. Most readers will welcome the opportunity to preview the papers that will be read at the meeting but few realize that many problems are associated with early publication. First, the contributors must have their abstracts in the hands of the Program Committee by November 1 so that they may select the items for the program and give the Editor the abstracts and the order of presentation by December 1. The Editor then has two or three weeks to prepare the manuscript of the Proceedings for transmission to the printer. The proof can then be returned to the Editor shortly after mid-January and the "book" distributed early in February. Obviously, the papers presented will not be as "current" as those read when the authors had two extra months for preparation.

Graduate students, planning on June theses, may be able to prepare abstracts for January and papers for March but seldom can they have abstracts by November 1. The Program Committee must plan on open time during November and the Editor must be prepared to devote early December to this long manuscript. On the other side of the ledger, pre-publication may be worth these sacrifices. It is for the Association to decide.

The "Read By Title" papers are noticeably absent. Your Editor, even against advice of the members of the Publication Committee, decided to publish these "by title" and not by abstract. Except in exceptional instances where they are used as substitute papers, they are never read to the Association. Although it is not usual, they could be used to abuse the privilege of publication priority, being submitted in November and published in February. By not publishing them, two original publications received in April have been printed.

We present all of this discussion because this is our first and last chore at editing "prepublished" abstracts. The readers should recognize the problems of future contributors, program committees, and editors. Many of the problems of publication are just that clear if they should be presented by the editors, but editors prefer not to air their problems to their contributors, assuming responsibility for solving the editorial problems. Sometimes they smile inwardly at well-intended, but naive, advice just as a specialist in science or practice may recognize the naiveté of well-intentioned, but inept, advice. By and large, it has been 22 years of stimulation and pleasure with the thorns serving only as challenges.

Dewar (p. 100) investigated gingival debris, from patients with various degrees of periodontal disease, for the presence of tissue-destroying enzymes. While hyaluronidase, chondroitin sulfatase and gelatinase were always present, and hide-powder collagenase was present in most samples, native collagenase was present more commonly in patients with marginal gingivitis and periodontosis than in normal individuals. Attempts at isolation from pure cultures of spirochetes and fusiforms resulted in demonstration of only a slight enzyme activity. The author proposes that the enzymes produced in the saliva may be highly important in the breakdown of tissues during periodontal disease. Whether or not these destructive enzymes are derived from bacteria, or as the result of inflammatory reaction caused by bacteria, seems relatively unimportant.

Rovelstad, Geller, and Cohen (p. 107) established base line data on human salivary hyaluronidase activity by intensive study of young adult males over a 6-week period and by evaluation of salivary hyaluronidase in a large group of Naval recruits at weekly intervals for 3 weeks. Using these data and technics, they could find no correlation between caries and salivary hyaluronidase, but greater salivary hyaluronidase activity was

associated with increased degrees of gingivitis, and there was greater salivary hyaluronidase activity in mouths with poor oral hygiene (p. 114). These rather extensive studies of controlled groups of young men offer valuable data.

Changes in the major salivary glands of the rat and mouse have been noticed in stressed animals and animals injected with cortisone. Shklar, Glickman, and Turesky (p. 119) administered cortisone to mice and then examined their salivary glands and salivary glands of the control mice by staining with hematoxylin-eosin and periodic acid-Schiff. The administration of cortisone increased the intensity in the staining of polysaccharides and the acinar epithelium of the submaxillary gland but the authors are unable to explain the manner in which this change was brought about. While it is interesting that histochemical studies, and other methods, often reveal changes in the tissues, there is still a need for a more thorough understanding of the changes which are identified microscopically.

Derksen and van Haeringen (p. 125) investigated the protrusive movements of articulators with a joint and incisal pin. They have classified the articulators and investigated the various types. It is their opinion that articulators of certain types reproduce the movements of the human lower jaw, the only difference being that the length of the movement line of the articulator is considerably greater than that of the human jaw. For this reason, in setting the articulator, care must be exercised in choosing the angles.

Wilkinson and Haack (p. 136) studied the fatigue characteristics of silver amalgam. It is their belief that this property of the material is of much greater importance than the static strength which has been reported previously. The results of their study contribute to the total knowledge of the properties of silver amalgam and also to knowledge in the field of physical testings that is associated with silver amalgam. Suitable technics to produce specimens for use in fatigue tests were developed. Application of the method showed that the highest fluctuating stress which the silver amalgam tested might be expected to withstand to be only 22 per cent of the maximum crushing strength. This study again indicates the need for continued investigation of the physical properties of dental materials in spite of the accumulated knowledge of the past.

Bennett (p. 144) examined 50 anesthetic needles which had been exposed to the atmosphere after sterilization and a similar group of 50 anesthetic needles covered by absorbent cotton after sterilization. In 10 of the exposed needles, and 2 of the cotton-covered needles, bacteria were found after 15 minutes of exposure. The author points out the potential danger of leaving the needles exposed to the atmosphere after their sterilization.

The injection of glucose and mannose labeled with C^{14} outlined the enamel and dentin formed at the time of injection (3 days after birth). The distribution of the two substances was indistinguishable. Kumamoto and Leblond (p. 147) found that the radioactive carbon of glucose was taken up by the pre-enamel matrix and it persists into the zone of calcification in young and maturing enamel. However, with time, the radioactivity is no longer detectable. In the dentin the carbon glucose is taken up by the predentin, remains in the dentin, and persists throughout a 6-month experimental period. It is suggested that the radioactive glucose in the dentin is bound with collagen which is a stable element.

Everett, Jump, Holder, and Williams (p. 162) investigated a ridge at the bifurcation of lower first molar teeth under the dissecting microscope by ground and decalcified sections. This structure, named the intermediate bifurcational ridge, was noted in 73 per cent of 328 lower first molars. They also observed the frequency of bucco- and lingual bifurcational ridges which appeared to guard the entrance to the bifurcation of the lower first molar and of the bifurcational buccolingual concavities convex to these planes. The structure appears to be formed of cementum on the basis of a structure in the dentin.

Muhler (p. 170) demonstrated that stannous chlorofluoride and stannous fluoride significantly reduced dental caries experience in salivariadenectomized animals. When the amount of fluoride as sodium fluoride in the drinking water was increased the dental

caries experience decreased but a similar increase in the stannous fluoride concentration of water did not effect caries. Analysis of the data from a study planned to compare the anticariogenic effect of two stannous fluoride preparations indicated pronounced differences in fluoridated water intake which may have affected the caries activity. It is suggested that food intake, water intake, and feeding habits be carefully controlled in experimental dental caries studies. In addition to this, it is essential that one consider whether a study is designed as an experiment on the initiation of caries or on the progression of dental caries.

Meyers, Walter, and Green (p. 175) have isolated a bacteriophage specific for a *Lactobacillus casei* from oral washings. It is suggested that this bacteriophage might be useful for identification of lactobacilli. The possibility of relationship between oropharyngeal infections and bacteriophage, and the possible development of a virulent strain of oral organisms from avirulent strains by exposure to bacteriophage is suggested.

H. B. G. R.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH #36

Abstracts of Papers to Be Presented
Hotel Statler, Detroit, Mich.
March 20-22, 1958

Compiled by Hamilton B. G. Robinson, D.D.S., M.S., Editor*

I. Development and Calcification. March 20, Forenoon, Section A.

1. Comparative Studies of Calcified and Skeletal Tissues. I. Limulus Cartilage.—*Albert Fine and Philip Person, VA Hospital, Brooklyn.* The cartilaginous endoskeleton of an invertebrate, Limulus, in which cartilage replacement by bone cannot occur, was studied morphologically and biochemically to determine whether significant differences exist between it and vertebrate and mammalian cartilages which do participate in endochondral ossification. Histologically, cartilage from young Limulus specimens is very similar to early embryonic human limb cartilage. In adult specimens, the cartilage bears a striking resemblance to mature human hyaline cartilage. Biochemical studies of terminal oxidizing enzyme systems in cell-free adult cartilage homogenates were negative. However, homogenates from young animals contained a cytochrome oxidase capable of direct oxidation of reduced cytochrome c. The oxidase was inhibited 62 per cent by 10^{-4} M cyanide and 32 per cent by 10^{-4} M azide. Succinic dehydrogenase and a succinoxidase complex which was inhibited 100 per cent by equimolar malonate were also found. This marks the first direct detection of cytochrome oxidase and succinoxidase activity in cartilage from any source. Certain experiments suggested the possible presence of an inhibitor, or inhibitors, of aerobic oxidative metabolism in Limulus cartilage. Preliminary experiments to test this assumption have revealed that homogenates of lyophilized adult Limulus cartilage inhibit oxygen uptake by rat heart muscle cytochrome oxidase.

2. A Longitudinal Study of Tooth Calcification and Root Resorption.—*Elizabeth A. Fanning. The Forsyth Dental Infirmary for Children* and *The School of Public Health, Harvard University, Boston.* This investigation was designed to establish norms, including measures of individual variation, of tooth calcification and root resorption in children with nonmutilated dentitions. In addition, the effects of extraction and agenesis on dental development were studied. The data available for analysis consisted of lateral skull and jaw radiographs, as well as intraoral radiographs of the anterior teeth. Calcification and resorption of all mandibular teeth, except the incisors, were studied from birth to 11.5 years. Calcification of the permanent incisors and also resorption of the deciduous incisors were assessed between 4 and 11.5 years. From the total series of children, sufficiently complete data were available for 48 boys and 51 girls. Calcification and resorption stages were defined. The age scale used was the logarithm of conceptional age and the means and standard deviations of ages of attainment were expressed according to this scale. Great similarity of the standard deviations was revealed not only for all stages of individual teeth but, also, for different classes of teeth in both jaws of both sexes. This suggests that a similar mechanism of growth applies to different teeth.

*The additional members of the Program Committee, Ned B. Williams, R. F. Sognnaes, and Dan Y. Burrill, gave invaluable assistance in preparation of these abstracts. Due to late changes, the papers may not, in all instances, be presented in the same order as they appear here.

3. Enzyme Patterns Associated With Calcification.—*M. S. Burstone, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* There is some difference of opinion in the literature as to the sites of enzyme activity in both normal and abnormal calcifying structures. The variance of previous work may relate to selective inactivation produced by different technics of fixation, as well as diffusion artefacts produced with some of the older technics. In the present work, carefully fixed (frozen-dried) undecalcified specimens of immature rat, mouse, and hamster teeth and bones were sectioned at 8 microns, dry mounted on slides and subjected to azo-dye procedures which reveal certain hydrolytic enzymes and permit precise histochemical localizations of alkaline phosphatase, acid phosphatase, and phosphamidase. The substances were phosphates and phosphoamides of complex arylamide derivatives of hydroxynaphthoic acid. Although the stratum intermedium exhibited strong alkaline phosphatase activity, the adjacent ameloblasts were unstained even with prolonged incubation periods. The odontoblastic zone was reactive, especially adjacent to areas of dentin apposition. The subjacent pulp tissue exhibited a concomitant activity. Several transplanted mouse osteogenic sarcomas revealed intense activity of tumor cells adjacent to sites of osteoid formation. Although fibers entering bone matrix stained, the matrix, per se, did not stain. The acid phosphatase and phosphamidase procedures (pH 5.2) revealed intense staining of osteoclasts and bone matrix.

4. Calcification and Esterase Localization in Developing Dentin.—*William B. Savchuck and Marvin S. Burstone, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* Hamster teeth were studied by a coupling of technics, the same section being microradiographed after the histochemical procedure. Undecalcified 10-micron sections of developing molars from the frozen-dried heads of newborn hamsters were stained for esterase (naphthol AS-MX procedure), following which the sections were photographed with soft x-ray. Correlation of the radiographic findings with sites of enzyme activity appeared to be excellent. Regions of actively calcifying dentin, bordering on the predentin, were relatively less densely calcified radiographically and showed the most intense esterase reaction. These findings corroborate previous reports associating esterase activity with calcification of dentin.

5. Dental Organic Matrices Studied by High Resolution Microradiography.—*Richard C. Greulich, University of California, Los Angeles.* The microradiographic method has been applied most extensively to the study of mineral distribution in bone and dental tissue, utilizing x-radiation in the wave length region, 0.1-5.0 A.U. By combining special instrumental conditions with a simple modification of sample preparation, the method now permits the study of organic mass distribution, with a degree of resolution exceeding 0.2 micron. Normally growing rat teeth and normal and scorbutic guinea pig molars were demineralized in disodium-EDTA (pH 7.4) following alcohol-formol fixation. The tissues were sectioned in paraffin at 6 microns. Microradiography of the sections was carried out within an enclosed, specially designed, high-vacuum x-ray tube, operated at 1.5 kv. and 1.0 Ma. (effective wave length range, 8-20 A.U.). The resultant microradiograms revealed a number of features in the dental organic matrices including the presence of a greater dry mass of organic material (a) in newly-formed enamel matrix than in older enamel matrix, (b) in newly-formed enamel matrix than in dentin matrix, and (c) in peripheral dentinal matrix near the dentinoenamel junction than in the remaining dentin. Other observations indicate the addition of dry mass to the dentin matrix at

the predentino-dentinal junction; the prominence of the terminal bar apparatus in the formation of enamel rods; and the abnormally low mass of pulpar bone matrix occurring in scurvy.

6. Effect of Synthetic Amino Acid Diets Upon the Bones and Teeth of Rats.—*L. A. Bavetta, B. Ershoff, and S. Bernick, University of Southern California, Los Angeles.* Young weanling rats were given synthetic amino acid diets for a period of 3 weeks. At the end of the experimental period, the animals were sacrificed and the tibias as well as the heads were placed in an alcoholic Bouin's solution. The specimens were decalcified, dehydrated, and infiltrated for nitrocellulose, embedding in the routine manner, sectioned at 20 microns, and the cut sections were stained with hematoxylin and triosin, periodic acid-Schiff method, and Pearson's silver impregnation technic. Microscopically, there was a widening of the epiphyseal plate of the proximal head of the tibia with an increase in ground substance and disorganization of the cellular columns. Osteoporosis of both the interradicular and interseptal bone were noted. Carious lesions were observed in the dentin of many of the experimental animals.

7. Localization of Vitamin C in Dental Tissues of Scorbutic and Non-scorbutic Guinea Pigs.—*C. I. Mohammed, School of Dentistry, University of Puerto Rico, San Juan, P. R., and S. H. Yale and H. Jeffay, College of Dentistry, University of Illinois, Chicago.* This study is based on complete serial histologic sections through the pulps of mandibular incisors of 30 guinea pigs. Twenty animals were rendered scorbutic by the feeding of a vitamin C-free diet, and 10 guinea pigs were controls. The control animals received 3 mg. of vitamin C daily, intraorally. After 3 weeks, symptoms of acute scurvy became apparent in the experimental animals. All animals were then fed 1.5 mg. of ascorbic acid containing 11.55 μc of activity. The animals were sacrificed at intervals ranging from 2 hours to 96 hours. Sections were stained for vitamin C and counter-stained with hematoxylin and eosin. Radioautographs were made of the sections. Very little vitamin C could be demonstrated in the control animals. Up to 24 hours, in the scorbutic animals, the vitamin C was localized to the cells at the periphery of the pulp. These cells, which were low cuboidal in shape, appeared to be the original odontoblasts which had lost their morphology but not their potentiality to produce dentin, which was formed within 48 hours. There was an increased gradient of vitamin C uptake up to 48 hours. The odontoblastic layer is the most active site of vitamin C metabolism in the pulp.

8. The Effect of Ca Reduction on Skeletal Development in Echinarachnius Parma Embryos.—*G. Bevelander, College of Dentistry, New York University, New York.* Observations were made on the development of the embryos of *E. parma* in which the Ca content of the sea water in which these organisms develop was reduced without modifying the osmolarity of this medium. When the Ca content was reduced to one tenth the normal value, the embryos developed in a relatively normal manner for the first 24-36 hours except for the appearance of a skeletal system. When the Ca content was increased to 0.3 normal Ca content, development was relatively normal and skeletal elements were also fabricated. In an intermediate range (0.2 Ca), skeletal formation occurred in some instances but usually was delayed in appearance or suppressed entirely. When embryos were reared in sea water containing compounds such as protamine sulphate, a quaternary ammonium salt, or toluidine blue, all containing strong postively-charged molecules, the embryos developed but were devoid of skeletons. These

observations indicate (1) that a higher concentration of Ca is necessary for skeletal development than for metabolic processes permitting embryonic development. (2) The effect of strong postively-charged molecules in contact with the surface of the embryo through which ions are transferred inhibits or suppresses Ca transfer and, as a consequence, skeletal development. The additional observation that ions reaching the internal regions of the embryos in which the skeleton develops must pass through an ectodermal layer containing a sulphated polysaccharide suggests that this substance may be associated with cation transfer.

9. Effect of Carbon Dioxide Toxicity on Formation of Dentin.—*W. R. Stanmeyer, and C. T. G. King, U. S. Naval Medical Research Laboratory, New London, Conn., and R. A. Colby, and H. H. Scofield, U. S. Naval Dental School, Bethesda.* The toxicity of carbon dioxide and what effects this toxicity has on dentin formation and on changes in blood normals was investigated. Sexually mature male rats (Wistar-Hisaw), weighing between 250 to 300 Gm., were exposed to 15 per cent carbon dioxide for 15 days and sacrificed. To prevent the effects of blood and tissue changes due to anoxia, a 21 per cent oxygen concentration was maintained in the gas mixture. During the experimental period, temperature, humidity, and night-day light cycles were carefully controlled. A control group of rats gained an average of 64 Gm., the experimental animals showing only an 8.5 Gm. mean gain. Night activity was observed and no visual narcotic effect of carbon dioxide was seen. Histologic examination of the dentin of the incisor teeth showed accentuated pairs of light and dark lines, first noticeable between 220-250 microns from the pulp; these lines continued to the pulp. Even though serum calcium remained normal, serum inorganic phosphorus decreased. Blood carbon dioxide content rose to 100.56 volumes per cent. Blood sugar and muscle glycogen remained unchanged but liver glycogen was almost totally depleted. This was verified chemically and histologically. Parallel studies on 30 per cent carbon dioxide indicate that liver glycogen depletion is not a nutritional factor.

10. Peritubular Matrix in Dentin.—*Shosaburo Takuma, Tokyo Dental College, Tokyo, Japan.* Under the optical microscope, a transparent zone can be seen around the dentinal fiber. There has been much argument about the morphologic significance of this zone. The present study has been carried on to investigate the problem by use of both optical and electron microscopes. The transparent zone seen at the tubule borders in ground sections is actually a solid structure. This is thought to be a special matrix (peritubular), which is more highly calcified, more birefringent, and containing less organic matter than the ordinary intertubular matrix. It shows great variation in size and shape, however, according to the species of animal and the region within the dentin and, in some cases, it seems to be missing. The empty space around the dentinal fiber, which is usually seen in demineralized sections, may possibly be the result of destruction of the peritubular matrix.

11. A Technic for the Study of Internal Structure of Calcified Tissues.—*Donald E. Knapp, James K. Avery, and Emmett R. Costich, School of Dentistry, University of Michigan, Ann Arbor.* Freshly collected teeth and bones were subjected to ethylenediamine extraction in a modified Soxhlet extractor to remove all organic constituents. After extraction, the samples were washed in water, dehydrated in ascending series of alcohol, and then transferred to anhydrous ether. One series of specimens was impregnated with an acrylic

and another with an unsaturated polyester resin. After infiltration, the plastic was cured and the samples were decalcified with either citrate buffered formic acid or ethylenediaminetetraacetic acid. In one series of tissues, the distribution of the plastic representing the organic constituents and water was observed directly in the decalcified specimens. In the second series, the specimens were re-impregnated with gelatin and then sectioned on the freezing microtome. The resulting plastic framework representing the organic and water constituents was studied microscopically. Another series of anorganic tissues was impregnated with plastic containing a particulate dyestuff, decalcified, and subsequently re-impregnated with clear plastic. The internal structure of calcified tissues can be clearly revealed by this technic. By impregnation and decalcification a plastic skeleton was produced which represented the distribution of the organic matrix and water while the areas where plastic was absent represented the distribution of inorganic salts. By impregnation with plastic containing a dyestuff into the spaces occupied by organic substance and water and then by decalcification and further re-impregnation with clear plastic into the spaces previously occupied by the inorganic salts, a contrasting picture was gained which revealed the positional relationship of the inorganic to the organic and water-filled spaces.

12. Analysis of an Anomalous Oculodentofacial Pattern in Newborn Rats Produced by Maternal Hypervitaminosis A.—*Frederick M. Deuschle, Jean F. Geiger, and Josef Warkany, Departments of Anatomy and Pediatrics, and the Children's Hospital Research Foundation, College of Medicine, University of Cincinnati, Cincinnati, Ohio.* Among teratogenic agents now available, maternal hypervitaminosis A produces, in newborn rats, exencephaly, hydrocephaly, spina bifida with meningocele, eye defects, cleft palate, shortening of the jaws, etc. Among anomalies produced by this method, and which has not been observed in newborn rats exposed to other teratogenic agents, is extreme exophthalmos and open lids associated with gross failure of development of the orbit. Serial sections of specimens with this syndrome were studied and anomalies of certain derivatives of the mandibular arch were found. In some instances the mandible and maxilla were connected posteriorly by a bony bridge, resulting in ankylosis. In the maxillary and infra-orbital regions, a cartilaginous structure, probably a persistent cartilaginous palato-quadrate bar (a normally regressive embryonic structure), was seen. Both jaws were shortened proportionately so that no anteroposterior malrelationship between the upper and lower jaws was evident. Both the mandibular and maxillary molar teeth were absent or reduced in size in varying degrees. The dentofacial anomalies of this syndrome and the anomalies of the "riboflavin deficiency syndrome" will be compared.

II. Microbiology. March 20, Forenoon, Section B.

13. Bacteriologic Census of Human Saliva.—*Robert L. Richardson and Marion Jones, Department of Bacteriology, College of Medicine, State University of Iowa, Iowa City.* Each of 14 adult subjects contributed 4-6 ml. of nonstimulated midmorning saliva on 10 separate occasions during a 5-month period. Specimens were serially diluted in saline, and appropriate media were inoculated as pour or spread plates. The average of duplicate plate counts was made following 4 days at 37° C. under aerobic or anaerobic atmospheres. The group means for 14 bacterial groups was expressed as high, intermediate, and low populations. High population means ($n \times 10^6$ per ml. saliva) were: total

anaerobes, 110; total aerobes, 40; total streptococci, 18; *Veillonella*, 17; *Streptococcus salivarius*, 11; starch hydrolyzers, 5; hydrogen sulfide-producers, 2; and Neisseria 2. Intermediate population means ($n \times 10^3$ per ml. saliva) were: Fusobacterium, 56; lactobacilli, 35; salt-tolerant micrococci, 5; and Leptotrichia, 3. Low populations ($n \times 10^2$ per ml. saliva) were: Candida, 2; coliforms, 1; and Bacillus, 0. Standard deviations varied from 50 to 100 per cent of the category mean. No statistical differences were found between vegetarians and non-vegetarians (3 subjects) for any of the 14 categories. Subjects with statistically significant high counts in one or more of the categories of population counts of micrococci, Neisseria, Leptotrichia, Fusobacterium, and hydrogen sulfide-producers, had varying degrees of gingivitis and calculus deposits, whereas subjects with significant low counts had clinically healthy gingiva and little or no calculus.

14. Microscopic Observations of Leptothrix Racemosa.—*William A. Nolte, The University of Texas Dental Branch, Houston.* This is a preliminary report of a study on the club-shaped bodies located about the terminal ends of filaments of the microorganism, *Leptothrix racemosa*. Numerous isolations of this microorganism were made with a micromanipulator from gelatinous microbial material scraped from teeth. Each isolation was placed in culture media on a slide and sealed in place between a cover glass and the slide. Each slide was incubated at 37° C. in a warm stage placed on a phase contrast microscope. Observations and photomicrographs were made. Single and clumps of racemosa filaments were studied. Observations indicate that the club-shaped bodies have a definite orderly arrangement about the filaments and appear to be attached to them by short stemlike structures. A series of photomicrographs show that the club-shaped bodies, during incubation, first swell and then bud off spherical bodies which are set free in the medium. These spherical bodies, although free, seem to form a pattern about the club-shaped bodies from which they were produced; this gives added support to their origin. The free spherical bodies have been observed to divide by binary fission. No filaments were observed to develop from these bodies. With age, the club-shaped bodies become ghostlike and the parent filament breaks up into segments, resembling large rod-shaped bacteria.

15. Growth and Enzymatic Activities of Oral "Actinomyces."—*William H. Bowen and Marion N. Gilmour, Eastman Dental Dispensary, Rochester, N. Y.* Eight strains (Group I) of actinomyces-like organisms have been isolated from calculus or gingival scrapings of 8 subjects, and their characteristics compared with the characteristics of 4 identified Actinomyces (Group II). Six of Group I are aerobic, and morphologically consist of gram-positive branching filamentous cells. Two of Group I and all of Group II are anaerobic, and include both diphtheroid-like cells and branching filamentous cells. No aerial hyphae have been observed. All strains exhibited metachromatic granules with the Laybourn stain, and none were acid fast. Growth media include: casitone yeast extract medium described by Howell and Pine; brain-heart infusion (Difco) supplemented with 1 per cent yeast extract; and a 1:1 ratio of brain-heart infusion and thioglycollate broths supplemented with 0.1 per cent starch and 0.2 per cent yeast extract. All strains are catalase negative, do not hydrolyze gelatin, do not produce H_2S, do not produce acetylmethyl-carbinol from glucose, and produce acid but no gas from glucose and arabinose. The anaerobic strains produce acid from starch, the aerobic strains do not. There is no systematic pattern in the fermentation reactions on the other carbohydrates tested. Acid phosphatase is found in washed suspensions of all strains and in their growth media.

16. The Growth Characteristics of an Oral Gram-Positive Filamentous Organism.—*Marion N. Gilmour, Patricia Hunter, Eastman Dental Dispensary, Rochester, N. Y., and G. H. G. Davis, Birmingham University, Birmingham, England.* Little is known of the organisms morphologically resembling Bulleid's *Leptothrix buccalis,* Bibby's Group I or *Leptotrichia buccalis,* and Morris' Leptotrichia. Fifty morphologically similar strains have been isolated in pure culture from calculus, materia alba, and carious lesions. In any one colony, there are 3 types of cells: bacillus-like bodies each attached to one or more filaments of varying lengths, long filaments, and bacillus-like bodies. The predominating cell type in smears is a 10-100 microns long filament attached to a bacillus-like body twice its width. Branching is not generally seen, although bizarre Y forms have been encountered. All isolated strains grow well on brain-heart infusion medium supplemented with 0.2 per cent yeast extract, and optimal growth occurs in conditions of slightly reduced oxygen tension. Little growth occurs at normal room temperature. Upon initial isolation, pour plate colonies resemble a tight ball of hair with extremely filamentous edges. Microscopic observations of developing single cell cultures indicate that reproduction occurs as follows: The filaments increase in size, septate, and shortly thereafter breakage occurs at the septa to form a zigzagged row of bacillus-like bodies. Each bacillus-like body gives rise to first one and then more filaments, and the secondary filaments reproduce as described above. All strains are catalase positive, and hydrolyze starch. Of 20 strains tested, all hydrolyze hippurate, and esculin, all produce acetyl-methyl-carbinol from glucose, none produce indole, none liquefy gelatin, and all produce acid but no gas from glucose, sucrose, and fructose.

17. Location of Microorganisms in Experimental "Fusospirochetal" Lesions in Guinea Pigs.—*H. A. Hunter and E. M. Madlener, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada.* There is some lack of agreement as to the location of microorganisms in the tissues of "fusospirochetal" lesions. Subcutaneous lesions were produced in the groin of guinea pigs by inoculation with known mixtures of "fusospirochetal" organisms. The resulting abscesses and cellulitides were prepared for histologic study by using 3 fixatives, formalin, Bouin's and absolute alcohol solutions. A section of each was stained with hematoxylin and eosin, Levaditi's and Krajian's methods. Examinations were made of abscesses from 2 to 15 days' duration, 2 abscesses 35 and 40 days old, and 1 cellulitis of 3 days' duration. The formalin fixation was the best for seeing microorganisms. No definite layering of different organisms was seen in either the circumscribed or spreading lesions. In the lesions produced by a known combination of microorganisms that did not include spirochetes, there were numerous structures that closely resembled spirochetes, but which were fragments of reticular fibers.

18. Some Studies on the Nutritional Requirements of the Fusobacteria.—*Robert R. Omata, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* The requirements for growth factors were determined for several oral strains of *Fusobacterium nucleatum* in a basal medium consisting of vitamin-free casein hydrolysate, glucose, K_2HPO_4, KH_2PO_4, L-cystine, DL-tryptophane, inorganic salts, 8 vitamins and nitrogen bases. Cultures were incubated in anaerobic jars under an atmosphere of 5 per cent carbon dioxide and 95 per cent nitrogen, at 37° C. for 96 hours. By the omission process, pantothenate was found to be essential for growth in the range of 0.2 to 20 μM per 10 ml. Pantothenol, pantotheine, and CoA were also active. Adenine and hypoxanthine were required as nitrogen

bases in the range of 10 to 100 μM per 10 ml. A purine precursor, 4 amino-5-imidazole carboxamide, was effective in the partial replacement of the purine requirements. Xanthine, guanine, and uracil played no essential or stimulatory nutritional role.

19. The In Vitro Effects of Antibiotics on Oral Spirochetes.—*Edward G. Hampp, American Dental Association, at the National Institute of Dental Research, and Robert J. Fitzgerald, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* Three strains each of *Borrelia vincentii, B. buccale,* and a small oral treponema were employed in a tube dilution assay (J. D. Res. 31: 20, 1952). The inoculum consisted of 4-day-old cultures of the respective organisms diluted to give the following average viable counts per milliliter of the test mixture: small oral treponemas, 2.0 million per ml.; *B. vincentii,* 1.8 million per ml.; *B. buccale,* 0.5 million per ml. Inhibitory end points were determined by visual inspection of turbidity after 5 days of incubation at 37° C., and by dark-field examinations for relative numbers and motility. Potassium penicillin G inhibited all of the strains at 0.02 unit per ml. or less, celesticetin at 5.0 mcg. per ml. or less, and oleandomycin at 0.3 mcg. per ml. or less. Anisomycin, an antibiotic which inhibits monilias, trichomonads, and endamoebae, was without effect at 100 mcg. per ml. Polymyxin, which is primarily effective on gram-negative bacteria, was inhibitory at 25 mcg. per ml. The concentrations of these drugs at which spirocheticidal activity was demonstrable were usually 4 to 8 times the inhibitory end point. Novobiocin showed the greatest individual variation in effectiveness. The inhibitory growth concentration per milliliter for the small oral treponemas ranged from 0.6 to 6.0 mcg., while none of the treponemas was killed by 40 mcg. per ml. Inhibitory concentrations for *B. vincentii* ranged from 0.2 to 1.0 mcg. per ml. while the spirocheticidal levels ranged from 0.6 to 40 mcg. per ml. *B. buccale* was inhibited and killed by novobiocin at less than 0.15 mcg. per ml.

20. Infectivity of Oral Spirochetes in Cortisone-Treated and Germ-Free Guinea Pigs.—*Robert J. Fitzgerald, National Institute of Dental Research, Edward G. Hampp, American Dental Association, at the National Institute of Dental Research, and Walter L. Newton, National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service, Bethesda.* Four groups of 6 guinea pigs were injected with single pure strains of oral spirochetes. Three animals in each group received the organisms intramuscularly and 3 received intraperitoneal injections. The organisms were 2 strains of small oral treponemas and 2 strains of *Borrelia vincentii.* The inoculum was 1 ml. of a 3-day broth culture containing between 1.5 and 2.5 million viable spirochetes per milliliter. Comparable groups of animals were pretreated with 3 daily subcutaneous doses of 10 mg. cortisone before injection of the spirochetes, and daily doses of 5 mg. cortisone for the following 2 weeks. At this time there was no gross pathologic evidence that any of the spirochetal strains were able to establish an infection in either series of animals. Since the germ-free animal is known to be susceptible to a number of normally innocuous microorganisms, pairs of germ-free guinea pigs were injected intramuscularly with pure cultures of oral spirochetes. Each pair received 0.5 ml. of one of the following strains: small oral treponema, *B. vincentii,* or *B. buccale.* A fourth pair received 0.5 ml. of a mixture of equal parts of all 3 strains. A group of conventional animals were similarly treated for controls. After one week there was no gross pathologic evidence of the establishment of infection in either group of animals. Cultures of the heart blood and tissues from the inoculation site yielded no viable spirochetes.

21. Isolation and Characterization of an Oral Spirillum.—*E. M. Madlener, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada.* From oral scrapings inoculated as drops on a Proske and Sayers blood agar plate containing sodium lauryl sulphate 0.001 per cent, a spirillum could be isolated in 9 out of 10 trials from a rhizoid-shaped spreading surface growth. The spirillum was gram negative, peritrichously flagellated, 8 to 15 microns long by 1 micron wide, having from 5 to 10 curves. Growth in thioglycollate broth was floccular and abundant after 2 days. Colonies on agar surfaces were irregular, surrounded by a film of spreading surface growth with a rhizoid border. Shake tube cultures showed lenticular colonies. Biochemically the organism was glucose +, sucrose +, lactose +, mannite –, H_2S –, indol –, nitrate +. Agglutination experiments using ethyl alcohol-treated antigens showed approximately the same titers for 6 of the isolated strains tested. Formalin-treated antigens were not identical and provided a method of differentiating strains. The biochemical and pathogenic properties of this oral spirillum were similar to those of *Spirillum sputigenum* (Miller) and *Spirillum minutissimus* (Prevot). However, agglutination experiments with 6 strains of each of the 3 organisms showed no cross agglutinations.

22. Bacteremia Associated With Dental Procedures in Severely Handicapped Children.—*John R. Mink, School of Dentistry, Indiana University, Indianapolis, and Cosmo R. Castaldi, School of Dentistry, University of Alberta, Edmonton, Canada.* A group of 35 severely handicapped children, ranging in age from 4 to 15 years, who underwent multiple dental procedures were checked for possible bacteremia resulting from these procedures. The treatment on all patients was carried out under general anesthesia. Five cubic centimeter samples of blood were obtained after nasotracheal intubation of the patients and before the dental procedures were started. Another 5 c.c. of blood was obtained after completion of the dental procedures and before the nasotracheal tube was removed. These samples were immediately placed in culture media bottles to be studied for the occurrence of any bacterial growth. Of the 35 cases, 30 were negative before treatment. Five were positive before treatment; 2 with *Staph. albus*, 2 with hemolytic *Staph. aureus*, and 1 with *Neisseria catarrhalis*. Twenty-eight cases were negative after treatment. Seven were positive; 3 with alpha hemolytic streptococci, 2 with *Staph. albus*, 1 with diphtheroids, and 1 with hemolytic *Staph. aureus* and *N. catarrhalis*. Of the 5 cases which were positive before treatment only 1 was positive with the same bacteria after treatment, while 2 others were negative, and the remaining 2 were positive with a different bacteria.

23. Indicated Associational Conditions in the Habitat of Mouth Protozoa.—*Wayne W. Wantland, Edna M. Wantland, John W. Remo, Illinois Wesleyan University, Bloomington, and D. L. Winquist, Bloomington.* The incidence of *Endamoeba gingivalis* and *Trichomonas tenax* in 80 patients, ranging in age from 6 to 60 years, was 40 per cent and 22.5 per cent, respectively. Seventeen and one-half per cent harbored both parasites. *E. gingivalis* was found most frequently in persons 41 to 50 years of age (69 per cent); *T. tenax* in persons 31 to 40 years (40 per cent). *E. gingivalis* was found in each of 7 cases of periodontal disease, 4 of which also revealed *T. tenax*. Both of these protozoan parasites were frequently demonstrated in samples taken from patients showing hyperemic oral mucosa. In patients showing good dental hygiene and classified as clean-normal, 13 harbored *E. gingivalis;* 5 harbored *T. tenax,* and 4 carried both protozoans. Persons harboring mouth protozoa, particularly *E.*

gingivalis, showed a greater number of carious teeth than persons free of these organisms. Cultures in modified Balamuth's media revealed 5 persons harboring *E. gingivalis* and 7 persons harboring *T. tenax* not observed in examinations of original samples. Best cultures were obtained in media adjusted to a pH of 7.4 to 7.8. Tests of pH on tooth surface, gingival line and tongue surface indicated a pH range of 7 to 8 to be most frequently associated with the presence of protozoan parasites.

III. Anesthesia and Radiation. March 20, Forenoon, Section C.

24. A Comparison of Analgesic Agents in Dentistry.—*Walter L. White, William J. Carter, and Harold R. Englander, Naval Dental Research Facility, Great Lakes, Ill.* A subjective method of testing (by questionnaire) was used to evaluate the effectiveness of various analgesic tablets in relieving postextraction pain following 1 to 4 extractions in male patients (average age, 18 years). Each patient was given coded tablets, all containing the same analgesic agent, and was instructed to return and complete a data form. Two thousand six hundred and twenty eight (2,628) questionnaires were completed. Of all patients, 33 per cent did not have postextraction pain and did not find it necessary to take any tablets. The composition of the tablets used and the percentage of pain relieved for each agent were as follows: N-acetyl p-aminophenol (5 gr.) —93 per cent; N-acetyl p-aminophenol (2.5 gr.), aspirin (3.5 gr.), and caffeine (0.5 gr.)—90 per cent; N-acetyl p-aminophenol (2.5 gr.) and acetyl carbromal (2.5 gr.)—88 per cent; aspirin (5 gr.)—88 per cent; thenylpyramine HCl (25 mg.), sodium salicylate (200 mg.), acetophenetidin (150 mg.) and caffeine (30 mg.)—87 per cent; aspirin (3.5 gr.), Phenacetin (2.5 gr.), and caffeine (0.5 gr.) —86 per cent; and placebo—75 per cent relieved. Although all analgesics were significantly more effective in the relief of pain than the placebo, no statistical differences were found among the various tablets. In certain respects, the pattern of effectiveness of the various analgesics in this study paralleled that of a previous one. This appeared to indicate that the subjective method of testing the effectiveness of various analgesic tablets is valid.

25. A Method for Determining the Efficiency of Local Anesthesia.—*George J. Petropoulos, Reginald G. Bickford, and Joseph A. Gibilisco, Mayo Foundation and Mayo Clinic, Rochester, Minn.* Recording electrodes were inserted directly into the gasserian ganglion of 48 cats. Stimulating electrodes were placed peripherally in the gingiva. Nervous impulses following electrical stimulation were recorded and analyzed in the usual electrophysiologic manner. A local anesthetic (brand of lidocaine hydrochloride or procaine hydrochloride), not containing a vasoconstrictor, was infiltrated into the soft tissue, the correct volume and concentration having been determined previously. Onset of anesthesia was apparent by the change in nerve conduction. The duration and intensity of the anesthesia could be evaluated with this method. The animals were divided into 3 groups. Group 1 received 2 minims of varying concentrations of either lidocaine hydrochloride or procaine hydrochloride; Group 2, 4 minims; and Group 3, 8 minims. Onset of anesthesia was more rapid with an increase in the volume of the solution injected, regardless of which drug was utilized. Duration of anesthesia was greater when a 2 per cent solution of lidocaine hydrochloride was used. This was especially noticeable in one group of experiments in which the duration was 2 or 3 times as long as in the groups receiving 1 per cent lidocaine or 2 per cent procaine. Two per cent lidocaine hydrochloride also produced the greatest intensity of anesthesia. This was consistent in all groups,

26. Tranquilizing Drugs in Oral Surgery: A Clinical Evaluation.—*Lucian Szmyd, Clarence M. McCall, School of Aviation Medicine, and Earl T. Enright, Base Dental Clinic, Randolph Air Force Base, Texas.* A clinical evaluation of promazine HCl and meprobamate in the control of postoperative sequelae in oral surgery was carried out. The subjects in this investigation consisted of 150 patients requiring the removal of an impacted mandibular third molar. A statistical analysis of the results was based on the chi-square test. When the results of ataraxic drug therapy were compared to placebo the following observations were made. No significant effect was noted on the postoperative sequelae of pain, swelling, or trismus (P .05). Tranquilizing drug therapy failed to influence the postsurgical narcotic demand (P .05). The number of days lost during postsurgical convalescence was increased significantly for patients on promazine HCl (P .05) and meprobamate (P .01) therapy. The incidence of untoward reactions was not significantly increased (P .05). On the basis of these findings, the use of promazine HCl or meprobamate cannot be recommended for the control of postoperative sequelae in oral surgery.

27. An Investigation of Neural Impulse Transmission in Human Teeth.—*Robert Rapp and James K. Avery, School of Dentistry, University of Michigan, Ann Arbor.* Freshly extracted teeth were sectioned. One series was sectioned for microscopic study and a second series was bisected to expose the pulp chambers to reagents. Each series was then divided into 5 groups, 3 experimentals and 2 controls. The first group was treated with a 10^{-6} M solution of di-isopropyl fluorophosphate to inhibit the activity of nonspecific cholinesterase and then incubated with acetylthiocholine to reveal the sites of activity of the specific cholinesterase. The second group was incubated in a substrate of butyrylthiocholine to locate the sites of the nonspecific cholinesterase. The third group was incubated in acetylthiocholine without previous treatment by an inhibitor in order to indicate the presence of both the specific and nonspecific cholinesterases. The tissues of group 4 were incubated without a substrate while those of group 5 were treated with di-isopropyl fluorophosphate to inhibit the nonspecific cholinesterase and then incubated in the substrate butyrylthiocholine which reacts with only the nonspecific enzyme. All groups were then treated with ammonium sulfide to produce a brown precipitate at the sites of cholinesterase activity. The sections were mounted and the series of bisected teeth were decalcified, embedded in paraffin, sectioned and mounted. The sites of specific cholinesterase activity were concentrated along the coronal aspects of the pulp chamber in the area of the parietal layer of nerves as well as along the course of the pulpal nerves. Specific cholinesterase was found in the Tomes' fibers in the dentin. Nonspecific cholinesterase was observed throughout the pulp and appeared to be concentrated along the nerves and blood vessels. Deciduous teeth revealed less intensity of staining than permanent teeth. Neither groups of control tissues exhibited staining in the pulp tissue.

28. Palate Contour as a Limiting Factor in Intraoral X-Ray Technic.—*John H. Barr and Poul Grøn, Tufts University School of Dental Medicine, Boston.* Two hundred study casts made from impressions of adult mouths were surveyed to record contour lines over the palate area at 2 mm. intervals above the occlusal plane. This permitted study of the range of configurations of the palate in certain specified directions from each of the maxillary teeth, these directions being related to the desired locations for the placement of intraoral x-ray films. Analysis indicates that if any periapical structure is to be recorded beyond the ends of the roots of the teeth, little selection is available in the placement of the palatal edges of intraoral films behind maxillary teeth. The film

placement of advantage and the optimum direction for angulation of the x-ray beam have been determined to provide, for each film area, a reasonably correct orientation of structures with minimal dimensional distortion. Further improvement in intraoral technic appears possible through modification of the size and shape of intraoral films.

29. Natural Head Position. A Prerequisite for Satisfactory Interpretation of Cephalograms.—*Coenraad F. A. Moorrees and Martin R. Kean, The Forsyth Dental Infirmary for Children, Boston.* A method was developed to take cephalometric radiographs in natural head position, the eyes looking in a mirror, the head unsupported, but self-aligned to keep the distance between the midsagittal plane and film constant. A true vertical wire, directly in front of the cassette and reproduced on each film, permitted registration of head position in space. Natural head position was found to be remarkably constant in a group of 61 female students, examined twice with a 1-week interval. The standard deviation of head position determined by measuring the angle sella turcica-nasion and the vertical on each set of radiographs is 1.54 degrees, and the correlation coefficient of the angles SNV at the two examinations is + 0.85 ± 0.04. The biologic variation of various reference lines in the cranium (S.D. ranging from 3.55 to 6.69 degrees) was greater than the variation in registration of head position ($P < 0.01$). The line of reference used in the analysis of cephalograms should reflect the orientation of the head in space. This can be accomplished only by a reference line outside the head. A vertical extracranial line has been shown to be preferable to the routine use of such intracranial lines as the Frankfort horizontal or sella turcica-nasion line.

30. Mandibular Movement in Children Studied by Rapid Serial Cephalograms.—*Milton Superstine, Robert E. Moyers, and Stuart Hunter, School of Dentistry, University of Michigan, Ann Arbor.* The purpose of this study was to ascertain the nature of extended mandibular movements in a group of 6-year-old children possessing acceptable occlusions. Mandibular movements beyond the movement from rest to the occlusal position have not been studied thoroughly, possibly because of a lack of an accurate method. Orientated serial cephalograms taken at one-half second intervals were utilized. The instantaneous centers of rotation were located between each of the serial opening and closing films by the method of Reuleaux. The centers of rotation for the various movements and for different children were then evaluated and compared. This study supports Bennett's original findings that the center of rotation about which the mandible may be regarded as moving at any instant has a position outside of the condyle and is capable of considerable variation depending upon the nature and magnitude of the movement. Although the instantaneous centers of rotation exhibited extreme variation among the 30 children studied, the method revealed a trend in which the center shifted downward with continued opening and then moved upward on closure. In the sample studied, the centers of rotation for the opening and closing movements of the mandible showed extreme variation in their location during each opening and closing sequence in the same child. The findings support the view that mandibular movements in children are different from those in adults.

31. Scintillation Spectra From Dental X-Ray Machines Operated Under Clinical Conditions.—*H. D. Spangenberg, Jr., M. L. Pool, and R. P. Sullivan, Ohio State University, Columbus.* The x-ray output of a half wave, self-rectified commercial dental x-ray machine, operated under clinical conditions, was analyzed by means of a NaI scintillation crystal attached to a 100-channel

pulse height analyzer. The target crystal distance was varied from approximately 1 foot to 9 feet. The x-ray beam was collimated. (1) The maximum intensity occurred in the neighborhood of 28 kv. even though the kilovoltage dial adjustment on the machine was changed from settings between 45 kv. and 70 kv. and the milliammeter reading was changed between 2 and 10. (2) The maximum energy of the radiations increased with the increase in the kilovoltage settings of the machine. (3) The energies of the observed radiations for a given dial setting was approximately 2 times that of the setting. (4) The cut-off of the maximum energy of the radiation was not clearly defined. (5) Above the 60 kv. dial setting of the machine, the tungsten Kα line appeared. (6) Addition of external filtration did not alter the shape of the spectrum as much as anticipated, e.g., when the external filtration was increased to 2 mm. of aluminum, the intensity of the peak radiation was decreased by only 16 per cent and shifted but 7 per cent toward the high energy side of the peak.

32. Effects of Irradiation From Dental Unit on the Rat.—*Phillip E. O'Shaughnessy, Joseph C. Muhler, and Richard C. Webster, Indiana University, Indianapolis and Bloomington, Ind.* The purpose of this study was to investigate the effects of irradiation of the head from a dental unit at those dosages which would be used clinically over a 30-year life span. An evaluation of growth and histologic study of the reproductive organs, pituitary, salivary glands, and thyroid were made. Rats in Group I received a total of 444 r, those in Group II 888 r, and those in Group III were not irradiated and served as controls. Group I received the irradiation every 10 days and Group II every 5 days. The results suggested some effect of the x-rays on body growth, but the thyroid, testes epididymides, seminal vesicles, submaxillary and thyroid glands all were normal. The testes of all 3 groups showed normal spermatogenic activity and their epididymides contained motile spermatozoa. There was no difference in the number of interstitial cells or in the histologic appearance of the seminal vesicles.

33. Prenatal Effects of X-Radiation on Bones, Teeth, and Periodontium of Rats.—*Max Shapiro, California Institute of Dental Research and Cedars of Lebanon Hospital, Sol Bernick, University of Southern California School of Medicine, Lucian A. Bavetta, University of Southern California School of Dentistry, and Ben H. Ershoff, Western Biological Laboratories, Culver City, and Southern California School of Medicine.* Forty female rats were divided into 4 comparable groups of 10 each, mated, and time of pregnancy noted. One group served as nonirradiated controls. Remaining groups received a single dose of (a) 300 r, (b) 450 r, or (c) 600 r of total body irradiation on the eighteenth day of pregnancy. Living rats were born in all groups. The mortality rate during lactation increased with increased dosage of x-radiation. Surviving young from each litter were sacrificed at birth and on the fifth, tenth, and twenty-first days. Sections were prepared of long bones and jaws. The effects obtained were proportional to the dosage administered. A significant retardation in eruption of the first and second molars occurred in rats administered the higher doses of x-radiation. Degenerative effects were observed in the long bones which were related to the dose of x-radiation administered. At birth these consisted of destruction and loss of trabeculae and myeloid elements of the proximal head of the tibia. By 10 days of age, centers of epiphyseal ossification had appeared in offspring of rats administered 300 r as well as the nonirradiated controls. The offspring of the 450 r group, however, still exhibited osteoporosis, absence of myeloid elements, and lack of a center of ossification. By the twenty-first day, however, no differences were observed between surviving young in the various groups.

34. Penetration of I^{131} Around and Into Various Filling Materials.—*Harold L. Dute, Hines VA Hospital, Hines, Ill., Robert E. Going and Maury Massler, University of Illinois, Chicago.* Class V cavities were prepared in 245 freshly extracted teeth and filled with different materials. The teeth were immersed in a radioactive iodine solution (15-50 μc per c.c.) for 24 hours. Radioautographs were prepared under standardized conditions. I^{131} penetrated into the margins of all fillings in varying degrees. The order of penetration, from most prominent to least, was: self-curing acrylics, zinc phosphate cements, silicate cements, zinc oxide-eugenol cements, silver amalgam, gold inlay, red copper cement, copper amalgam, and gold foil. Radioiodine was adsorbed very strongly on the surface of copper amalgam, copper and zinc oxide—eugenol cements. It also penetrated into these fillings significantly. I^{131} was adsorbed strongly on the gold fillings but did not penetrate them significantly under the conditions of this experiment. I^{131} was poorly adsorbed on intact enamel, acrylic and silicate fillings. Since radiocalcium and other positively charged ions are markedly adsorbed on the surface of enamel and poorly adsorbed on metallic fillings (Armstrong and Simon, 1951; Wainwright, 1951), it is concluded that the ability to penetrate into enamel or fillings is in part a function of the charge on the ion.

35. Photomicrographic Technics Employing Broad Spectrum Light of Ultrashort Duration.—*William A. Curby, Tufts University School of Dental Medicine, Boston.* Essentially parallel light may be delivered to the microscopic specimen. By employing Huygens' principle it is possible to obviate the use of substage condenser systems, thus eliminating wave-length dependency inherent in refraction systems. The exposures used vary in duration from 0.5 to 1.0 milliseconds and through varying intensities to 300 watt seconds. Photomicrographs of gingival tissue illustrate color balance in various portions of the visible spectrum and some manifestations of ultraviolet illumination. The results of the technics discussed show that it is possible in unstained frozen sections to see: (1) clear differentiation between epithelium and connective tissue under any power, (2) possible visualization of intracellular spaces, (3) malpighian spine fibrils of the epithelium of gingival tissue, (4) clear visualization of bundlelike structures of connective tissue in normal and inflamed tissue, (5) irregularities in the connective tissue structure of pathologic tissue, and (6) salivary bacteria at high magnification.

IV. Oral Mucosa and Carcinogenesis. March 20, Afternoon, Section A.

36. Histochemical Investigation of Basement Membrane Changes in Experimental Oral Carcinogenesis.—*Alvin L. Morris, School of Medicine and Dentistry, Rochester, N. Y., and University of Pennsylvania School of Dentistry, Philadelphia.* The purpose of this study was to investigate changes in oral mucosa during neoplastic alteration. The hamster cheek pouch mucosa was studied in the normal state, when undergoing premalignant hyperplasia, and after the production of squamous-cell carcinomas, in response to repeated application of 9,10-dimethyl-1,2-benzanthracene. Bouin's fixed tissues were stained for reticular fibers according to the method of Foot. The periodic acid-Schiff (PAS) technic was employed with and without pretreatment with amylase, hyaluronidase, and pyridine. Staining with toluidine blue was carried out both before and after sulfation with and without pretreatment with hyaluronidase. Serially cut sections were subjected to Hale's iron absorption

staining procedure. The basement membrane was well visualized in Foot-stained and PAS-treated tissues. The PAS staining was not altered by previous treatment with amylase, hyaluronidase, or pyridine. The basement membrane exhibited metachromasia only after sulfation and this staining was not changed by hyaluronidase pretreatment. Hale's procedure did not selectively demonstrate the basement membrane. The basement membrane of epithelium undergoing preneoplastic hyperplasia exhibited increased thickness and staining intensity in PAS-treated tissues and those stained with toluidine blue after sulfation. This change was not evident in tissues stained for reticulum or those viewed in the phase contrast microscope. The basement membrane of epithelium contained in papillomatous tumors was less defined, thinner, and stained less intensely than that of normal or hyperplastic epithelium. In areas of cellular invasion, no basement membrane could be demonstrated.

37. Ultrastructural Observations on Oral Mucosa.—*John T. Albright and Reidar F. Sognnaes, Harvard School of Dental Medicine, Boston.* Ultrastructural changes associated with keratinization and desquamation have been explored by means of electron microscopy of the squamous epithelial lining of human and mouse oral mucosa. In the basal cell layers of the epithelium, the nuclei and nucleoli appeared round and uniformly dense, and the cell membranes were relatively smooth. In the cytoplasm of the basal cells, mitochondria were abundant, but diminished within the intermediary and superficial squamous cells. The so-called intercellular bridges, instead of being continuous, were represented by minute electron dense contact points, separated by oval and spheroid structures bordered by more delicate membranes. Toward the stratum granulosum, the homogeneous nucleolar material appeared to break up into electron dense satellites; the nucleoplasm became concentrated within folded areas along the nuclear surface; and, in near proximity to these foldings, especially at the nuclear poles, there appeared cytoplasmic complexes containing granules of various dimensions, mitochondrial fragments, and high-density keratohyaline granules. These granules were very large in the mouse, extremely small in man, and widely scattered through the cytoplasm of the intermediary and superficial cell layers. The squamous cells increased in surface area manifold near the oral surface and showed extensive prolongations extending deep into cytoplasm of adjacent cells. The amorphous ground substance was not conspicuous between these peripheral cells.

38. Further Studies on Experimental Oral Carcinogenesis.—*Paul Goldhaber, Harvard School of Dental Medicine, Boston.* Previous studies have revealed that the mouse labial mucosa, under conditions of desalivation, is capable of developing early carcinoma in approximately one year following repeated topical applications of 0.3 per cent 3-methylcholanthrene in acetone. Because of the high mortality rate due to the early development of skin tumors, it has not been possible to obtain more than a small number of survivors for this length of time. An attempt to overcome this problem by utilizing 0.5 per cent 7,12-dimethylbenzanthracene in mineral oil has yielded essentially the same results. Under these conditions, advanced squamous-cell carcinomas of the gingiva or labial mucosa were found in a high proportion of a small number of survivors. It appeared that this carcinogen was more potent for the oral mucosa, giving rise to gross intra-oral lesions and decreasing the latent period to approximately 8 to 9 months. The role of mucosal ulceration (obtained by periodically cutting down the lower incisors) in experimental carcinogenesis has been explored in the rat by means of fluorescence microscopy. It was found that topically applied carcinogen became localized in high concentrations in such ulcers despite an

adequate salivary flow. These findings encourage continued interest in the role of local oral trauma as a potential factor in the pathogenesis of human oral cancer.

39. Effect of Topical Application of Carcinogens on Palatal Mucosa of Hamsters.—*John J. Salley, Medical College of Virginia, Richmond, and Seymour J. Kreshover, National Institute of Dental Research, Bethesda.* The intraoral mucosa of experimental animals has exhibited a rather marked degree of resistance to locally applied irritants both of carcinogenic and noncarcinogenic nature. The cleansing action of the tongue and saliva has been credited with promoting this apparent immunity of the oral tissues when comparisons to other epithelia (hamster pouch and skin) have been made. The palatal mucosae, cheek pouches, and ears of 2 groups of hamsters were painted triweekly with 0.5 per cent 9,10-dimethyl-1,2-benzanthracene and 3,4-benzpyrene, respectively. Of 19 survivors, in the former group, 10 (54 per cent) showed evidence of early squamous-cell carcinoma of the palate while all of these animals had carcinoma present in the pouches and on the ears. Hamsters treated with benzpyrene presented a 16 per cent incidence of palatal malignancy, 94 per cent in the cheek pouches and 100 per cent on the ears. Latent periods were 16, 6, and 14 weeks, respectively, for the palates, pouches, and ears of the 9,10-dimethyl-1,2-benzanthracene group, and 46, 25, and 32 weeks, respectively, for these anatomic sites with benzpyrene. These data would tend to indicate that there is some resistance of the intraoral epithelium to carcinogenic action when compared to the epithelium of the pouch and of the ear; however, the fact that there was intraoral carcinomatous change leads one to conclude that the tongue and saliva do not contribute the complete mucosal protection previously attributed to them.

40. The Effect of Vitamin A Deficiency Upon Experimental Carcinogenesis.—*Nathaniel Rowe and Robert J. Gorlin, School of Dentistry, University of Minnesota, Minneapolis.* One hundred and twenty golden hamsters, 25 days of age, were housed singly in an air-conditioned room. They were fed Nutrena Rabbit Chow for 20 days, then weighed and assigned to 5 different groups so that each group contained an equal number of male and female hamsters of comparable weight. At 45 days of age, all hamsters were started on an experimental vitamin A deficient diet. Groups II, III, and IV received, in addition, a weekly supplement of 400 I.U. of natural vitamin A administered orally. All groups were fed ad libidum with the exception of Group III which was pair fed with Group I. The carcinogen, 0.5 per cent solution of 7, 12-dimethyl 1, 2-benzanthracene in liquid petrolatum, heavy grade U.S.P., was topically applied to the left buccal cheek pouch with a cotton swab 21 times over a period of 13 weeks in Groups I, II, and III. Groups IV and V were treated in an identical manner applying only liquid petrolatum. The animals were sacrificed by ether inhalation 7 weeks later. The cheek pouches were dissected out and paraffin sections, stained with hematoxylin and eosin, were prepared. Malignant tumors were produced in numbers to suggest that hamsters fed on a diet deficient in vitamin A show a higher tumor incidence than hamsters fed on a similar diet ad libidum but supplemented weekly with vitamin A. An even greater tumor incidence was observed in the hamsters fed on the diet deficient in vitamin A compared to hamsters supplemented with vitamin A whose caloric intake was restricted to that of the vitamin A deficient group.

41. Carcinogenesis in Duct-Ligated Submaxillary Salivary Glands.—*Samuel M. Standish and William G. Shafer, Indiana University School of Dentistry, Indianapolis.* A study was conducted to determine whether tumor

induction occurred more readily in excretory duct-ligated salivary glands. Controls, consisting of 58 weanling male albino rats of the Sprague-Dawley strain, were implanted with 7-12 dimethylbenzanthracene pellets in the right intact submaxillary salivary glands. The right excretory salivary duct was ligated in 60 animals and the carcinogen similarly implanted. Animals were sacrificed at 5, 6, 7, 8, 12, and 18 weeks following the operative procedures. Squamous metaplasia of striated duct epithelium was noted on histologic section in 31 control animals and 34 ligate animals. A diagnosis of epidermoid carcinoma (or carcinoma-in-situ) was made in 14 control and 13 ligate animals. Comparison of the 2 groups at the various sacrifice periods revealed no remarkable differences in tumor incidence, latent period, or degree of malignancy. Histologic features of duct ligation were observed in 31 control animals of which 27 had demonstrable concomitant squamous metaplasia, indicating that these features occurred as a duct blockage phenomena created by the metaplastic epithelium. Since the duct-ligated gland is reduced in volume with a relative increase in ducts per unit area of gland, the chances of an optimum mass of carcinogenic agent coming in contact with these ducts is thereby increased. The failure of the duct-ligated gland to be more susceptible to tumor induction therefore may represent a function of reduced metabolic activity of these cells.

42. Photographic Technics for Fluorescent and White Light Observations of the Same Tissue Section.—*Marjorie Houston, University of Illinois College of Dentistry, Chicago.* Although the fluorescence of carcinogenic hydrocarbons in its localization within tissue sections has been photographed, the exact tissue components which facilitate the penetration or absorption of the carcinogen cannot be identified without black and white photographs of the same tissue area when viewed in white light. Fluorescence photomicrographs of a 0.6 per cent solution of 3-methylcholanthrene in reagent grade benzene, applied to skin and oral mucosa of Swiss mice, were secured on Adox KB-17 film. The large Zeiss fluorescent lamp housing and microscope were used in the transmitted light technic. The choice of exciter filters in the lamp housing allowed either ultraviolet light or ordinary white light to penetrate the tissue section. The choice of barrier filters of the microscope allowed either the light of fluorescence or the ordinary white light to be observed and photographed. The insertion of a piece of opalized glass between the lamp housing and the microscope cut the intense glare of the white light so that an image of the tissue could be recorded on the sensitive emulsion of the same type of film as that used for autofluorescence and fluorescence.

43. Exfoliative Cytology as a Diagnostic Aid for the Detection of Oral Neoplasms.—*Henry C. Sandler, VA Hospital, Brooklyn, and S. Sigmund Stahl, New York University, College of Dentistry, New York.* This preliminary study indicates that the examination of smears of oral lesions may be sufficiently reliable to warrant its use as an aid in the early detection of oral carcinoma. In the 37 oral lesions studied there was agreement between the smear and biopsy findings in 33 cases (89 ± 1.9 per cent). The lesions of 4 of the normal smears were found to be positive on biopsy. Occasional false negative findings in histologic specimens, whether the specimen is a smear or a piece of tissue, are inevitable, for samples may be inadequate or may not be representative of the pathologic process. There were 2 suspicious smears which were negative on the first biopsy, and were subsequently proved positive after a second biopsy. Biopsy is the diagnostic procedure of choice for the detection of malignant neoplasms and since oral lesions are usually quite readily accessible, exfoliative cytology is no substitute for biopsy. However, there are many clinical situations in

which biopsies of oral lesions are not feasible, or not acceptable to the patient. The technic is well suited for follow-up of chronic oral lesions such as leukoplakias, and for the follow-up of patients who have been treated for oral malignancies in whom repeated biopsies are not warranted. Taking a smear of surface cells is so convenient a procedure and the results so reliable that the investigation and follow-up of comparatively innocuous appearing oral lesions may be encouraged by its wider application.

44. Possible Systemic Factors in Tongue Cancer.—*Norman Trieger, Irwin I. Ship, Grantley W. Taylor, and David Weisberger, Massachusetts General Hospital, Boston.* Of 108 patients, with complete histories, physical examination, and laboratory studies, admitted to the Massachusetts General Hospital with cancer of the tongue during the years 1946 to 1957, 76 per cent (82 patients) habitually drank alcohol in excess of 6 "shots" per day. Forty-eight (44.4 per cent) of these 108 patients had unequivocal evidence of hepatic cirrhosis, by physical and laboratory examination. An additional 6.4 per cent had histories which included acute yellow atrophy of the liver, benzol, naphtha, and cincophen toxicities, and severe nutritional deficiencies. Eighteen and five-tenths per cent of these 108 patients had pre-existing syphilitic glossitis and leukoplakia. In the 108 cases, 89.9 per cent smoked in excess of 1 pack of cigarettes per day. Seventy per cent of the total group had definite and distinct evidence of long-standing oral irritation in the forms of sharp, broken, or eroded teeth, or poorly-fitting dentures. Fourteen females of 108 patients had tongue cancer. Four of this group had hepatic cirrhosis, while a fifth female had Plummer-Vinson's Disease. The only factor common to the remaining 9 patients was their postmenopausal state, secondary to hysterectomy in 2 of the youngest females. The mucosal substrate altered by the effects of hepatic disease or the granulomatous degeneration of syphilis combined with the carcinogenic activity of tobacco and long-standing oral irritants, all seem to act in concert in the genesis of tongue cancer.

45. Histogenesis of Branchial Cysts.—*Surindar N. Bhaskar and Joseph L. Bernier, Armed Forces Institute of Pathology, Washington, D. C.* The branchial cysts are believed to represent cystic lesions of the neck which arise from the branchial cleft, pharyngeal pouch, or thymic duct remnants. The present study consists of a histologic and clinical evaluation of 460 cases filed at the Armed Forces Institute of Pathology. It showed that (1) branchial cysts occur most frequently between 20 and 30 years of age; (2) they occur in those sites where normally lymph nodes are seen; (3) 97 per cent of them show an epithelium-lined cyst with a periphery of lymphoid tissue (only about 3 per cent show an epithelium-lined cyst without lymphoid elements); (4) 97 per cent of these cysts really represent cystic-lymph nodes, they arise from the epithelial inclusions within the lymph nodes and not the branchial remnants, the remaining 3 per cent are epidermal cysts; and (5) branchial cysts recur repeatedly if treated by aspiration and drainage and require surgical excision.

46. Malignant Lymphoma of the Maxilla and Mandible.—*Ronald F. Steg, Robert J. Gores, David G. Pugh, and David C. Dahlin, Mayo Foundation and Mayo Clinic, Rochester, Minn.* The clinical and pathologic features of 47 cases of malignant lymphoma of the maxilla and mandible were studied. Any factors which could aid in diagnosis or improve results of treatment were emphasized particularly. Seventy-six per cent of patients were in the fourth to seventh decades of life, and there were 34 males and 13 females. Thirty-four

lymphomas (72 per cent) were in the maxilla, 12 in the mandible, and 1 in both bones. The majority of the maxillary tumors presented to some degree in the antra. Unlike bone tumors elsewhere in the body, swelling was the most frequent symptom. The average duration of symptoms was 10.6 months. Seventy per cent of these patients had some intraoral feature clinically. The roentgenologic findings were varied, with destruction being the most common feature. The destruction, however, had no definite diagnostic pattern and biopsy was necessary to obtain an accurate diagnosis. Forty-five per cent of the tumors presented a mixture of reticulum and lymphocytic cell types, 30 per cent were reticulum cell sarcoma, 22 per cent lymphosarcoma, and only 4 per cent were Hodgkin's disease. A most striking feature in the series was the high incidence of early erroneous diagnoses and mistreatment. Adequate radiation is the treatment of choice and should be instituted at the earliest possible time.

47. The Oral and Pharyngeal Pathology of Domesticated Animals.—*Robert J. Gorlin and Anand P. Chaudhry, School of Dentistry, University of Minnesota, Minneapolis.* Biopsies of 488 oral and pharyngeal lesions, obtained from 378 dogs, 44 cattle, 35 cats, 17 horses, 9 mules, 3 sheep, 1 burro, and 1 pig, were reviewed. These lesions include both neoplastic and non-neoplastic disease. Among the non-neoplastic group were pyogenic granuloma, actinomycosis, actinobacillosis, eosinophilic granuloma, trichinosis, globidiosis, teratoma, and odontoma. The benign neoplastic lesions were fibromatous epulis of periodontal origin, canine oral papillomatosis, noncanine papillomata, and the papillomata of bovine hyperkeratosis, chondroma, lipoma, adenoma, and nevus. The most common malignant oral neoplasm was canine melanoma which is in contrast to the squamous-cell carcinoma observed by Withers and Cotchin. Other malignant neoplasms found in different animals were squamous-cell carcinoma, basal cell carcinoma, carcinoma of the major and minor salivary glands, mast cell sarcoma, fibrosarcoma, chondrosarcoma, osteosarcoma, malignant lymphoma, rhabdomyosarcoma, transmissible venereal tumor, and "myelocytoma."

V. Tooth Structure and Chemistry. March 20, Afternoon, Section B

48. Electron Microscopic Studies of the Early Stages of Amelogenesis.—*Marie Ussing Nylen and David B. Scott, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* Four- and 8-day-old mice were perfused through the heart with a 1 per cent osmic-dichromate mixture. Immediately following perfusion the lower incisors were removed and placed in the fixative. The teeth were embedded in methacrylate and sections were cut with glass knives on a modified Spencer microtome, using thermal advance and automatic retraction. The apical part of the growing zone which could be cut without demineralization was studied. In the recently differentiated ameloblast almost all of the mitochondria are concentrated basal to the nucleus, and they are separated from the cell membrane by a band of dense fibrillar material. Rough surfaced endoplasmic reticulum and typical dense granules are distributed sparsely throughout the cytoplasm distal to the nucleus. There is little alteration in cellular detail until the beginning of enamel matrix formation. At this stage, the major change is a marked increase in the endoplasmic reticulum, which becomes closely packed and aligned more or less parallel to the long axis of the cell. In very thin sections, the first matrix formed, which looks amorphous in thicker sections, appears to be, in fact, composed of very fine fibrils.

49. Electron Microscopic Study of Anorganic Enamel.—*David B. Scott, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, and Fred L. Losee, Dental Division, Naval Medical Research Institute, Bethesda.* Previous information on the crystal structure of mature enamel has been derived largely from replicas of the surfaces of either etched ground sections or cleaved fragments. The crystalline elements have appeared long and ribbonlike and they have often borne distinct cross-markings. Since organic matrix fibrils have not been evident as individual structures in such preparations, it was of interest to explore the possibility that these markings might reflect the presence of fibrils within the crystalline formations. Ground sections of enamel were treated with ethylene diamine for periods from 15 to 90 minutes to render their surface layers anorganic. Positive carbon replicas and pseudoreplicas were made from these surfaces and they were shadowed with tungsten oxide. The latter material is amorphous and it was thus possible to subject the same areas which were photographed to electron diffraction. In many regions of these preparations large numbers of very small particles, often in chainlike configurations, were observed, while in other areas the long ribbonlike structures appeared intact. Typical apatite diffraction patterns were obtained from portions of pseudoreplicas in which only small particles were retained. These findings suggest that organic matter may have been removed from some of the long crystals, and that consequent fragmentation may have occurred.

50. The Separation of Human Enamel Into Fractions of Different Density.—*A. K. Bose, R. Q. Blackwell, and L. S. Fosdick, Northwestern University Dental School, Chicago.* When a tooth surface is etched with dilute acid, electron microscopy reveals that the cementing substance contains more organic material than does the enamel rod. On this basis the cementing substance should have a lower density than the enamel rod substance. Attempts were made to separate finely divided enamel on the basis of density. Pure human enamel was separated from dentin by mechanical means and fractionated on the basis of density by means of bromoform ethanol mixtures. The enamel was ground to 300 mesh by mechanical means, after which a 30 per cent slurry in distilled water was subjected to ultrasonic vibration at 3° C. for varying periods of time. The organic material was destroyed, as evidenced by an odor of burning hair and by an increase in total nitrogen in the water layer. The density of the powdered enamel was not appreciably altered. Since these ultrasonic vibrations partially destroyed the organic matter, procedures were developed to produce the finely divided enamel particles by mechanical means. The pure human enamel was powdered to 300 mesh after which it was separated by means of bromoform ethanol fractionation. The material, with density 2.60 or greater, was analyzed for calcium, phosphorus, carbonate, sodium, potassium, magnesium, and organic matter. The dense material was pulverized, fractionated at density 2.61, and analyzed 3 successive times. Each time a small fraction of lighter material was obtained. After each separation, the analysis of the dense material revealed a progressive decrease in organic material, calcium and phosphorus. There was a slight increase in sodium, potassium, magnesium, and carbonate. The amount of material with a density of less than 2.61 was too small for an accurate analysis.

51. An Amplifier-Graphic Recording Method for Measuring Enamel Permeability.—*Harold E. Brewer, Joseph C. Muhler, and Robert B. Fischer, Indiana University, Bloomington.* Previously described was a method for measuring enamel permeability which consisted of observing the conductance of

an aqueous phase within an enamel shell immersed in an electrolyte solution as a function of time of immersion. In order to increase the accuracy and the utility of this method, an amplifier-graphic recorder has been substituted for the conductance bridge. The amplifier-recording apparatus has the advantages of: (1) allowing direct objective measurement of conductance and, thus, permeability changes; (2) providing a continuous, permanent record of conductance changes occurring during any desired interval of time; and (3) good sensitivity to minute conductance changes. Sections of enamel prepared from human teeth and crowns of the mandibular cuspids of dogs were used in testing the amplifier-recording method of measuring enamel permeability. The experimental findings showed: (a) considerable random variability in the permeability of enamel of different teeth, (b) greater permeability of carious enamel as compared with that of noncarious enamel, (c) apparent decrease in enamel permeability with age, (d) similarity of the permeability of contralateral teeth, (e) that the effectiveness of aqueous fluoride solutions in decreasing enamel permeability is a function of the time of exposure of the enamel to the fluoride solutions, and (f) that the permeability-reducing effect of stannous fluoride solutions lasts for several hours.

52. In Vitro Study of Change in Contact Angle of Water on Tooth Surface Induced by Different Preservatives and Silicones.—*B. S. Savara, N. M. Phatak, University of Oregon Dental School, and J. A. Dement, Dement Laboratories, Portland.* The surface properties of silicones may be valuable in preventing caries action. Deposition of a silicone microfilm on tooth enamel was detected by change in the contact angle of water. This angle is a direct measure of free surface energy of a solid which affects the spreading behavior of liquids and was studied by the spheroidal segment technic. Horizontal and vertical dimensions of a drop of water on the enamel surface were measured with a fine scale ocular micrometer, and the angle was determined by the equation, $\theta = 2 \tan^{-1} H/x$, where θ is the contact angle, H is the height, and x is the radius of the drop at its base. Contact angle readings of 10 freshly extracted teeth were lower than readings of 10 formalin-preserved and 81 Zephiran chloride-preserved teeth. This difference is statistically significant. Of 8 different silicone products tested, 5 showed an increase in contact angle and hence an increase in free surface energy, and 3 showed a decrease. One silicone product was incorporated into 3 bland dentifrices which were applied to extracted teeth by brushing after control readings. Contact angle measurements indicated a reduction in the surface spreading action of water on enamel surface due to silicone film deposition.

53. Distribution of Tin and Fluoride Brushed on to Enamel.—*W. E. Cooley, The Procter & Gamble Company, Cincinnati.* Chemical methods were used to determine the amounts of tin and fluoride supplied to sound, pre-etched incisor chips by 0.1 M tin fluoride and 0.2 M sodium fluoride solutions. The chips were continuously brushed with nylon bristles while immersed in the treating solution. Enamel for analysis was obtained by dissolving layers of the treated chips in 2 M perchloric acid. Most of the tin taken up from tin fluoride solution is taken up in the first few minutes of exposure. The brushing treatment applies a nearly uniform tin-containing surface coat. Some tin penetrates the enamel slightly, but very little, if any, penetrates deeper than 0.02 cm. Fluoride is taken up from both tin fluoride and sodium fluoride solutions, but when each solution is at its natural pH, more is supplied by sodium fluoride. Since electron microscopy studies, reported previously, show that tin fluoride imparts more resistance to acid attack than does sodium fluoride,

the difference is attributed to the tin laid down on or near the surface. Fluoride taken up from either kind of solution penetrates the enamel much more deeply than does tin. There is no appreciable difference between the solutions with respect to depth distribution of the fluoride when treatment times are chosen to make the total amounts of fluoride equal. Surface effects of the brushing treatment and perchloric acid dipping were observed in electron micrographs.

54. Fluoride in Enamel, Coronal Dentin, and Roots of Human Teeth.—*Shin H. Yoon, Finn Brudevold, Eastman Dental Dispensary, Dwight E. Gardner and Frank A. Smith, University of Rochester, Rochester, N. Y.* Fluoride analyses were made of successive layers of enamel from the surface to the dentinoenamel junction and of coronal dentin from the junction inward. Layers from the external portion of the root and from pulpal dentin were also analyzed. Samples were pooled from 12 or more teeth obtained from 2 areas with 0.1 ppm and 1 area with 3.6 ppm of fluoride in the drinking water. In the enamel, the concentration of fluoride decreased from the surface inward, the outermost layer having 10 to 15 times more fluoride than the innermost enamel. Three to 4 times more fluoride occurred in the first layer of coronal dentin than in adjacent enamel. Subsequent layers of coronal dentin showed a gradual decrease but, approaching the pulp, the fluoride concentration increased markedly. Dentin facing the pulp had 10 to 15 times greater fluoride concentration than the dentin facing the enamel. The first layer of the root contained 2 to 5 times greater fluoride concentration than the outermost layer of enamel. The fluoride level decreased in subsurface layers of the root and increased again toward the pulp. The distribution of fluoride was the same in teeth from different geographical areas but the greatest concentrations were consistently found in teeth from the area with the greatest fluoride concentration in the drinking water.

55. Distribution of Strontium in Teeth From Different Geographical Areas.—*Finn Brudevold, Eastman Dental Dispensary and Luville T. Steadman, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Strontium was determined in successive layers of enamel of teeth from 7 geographical regions. Layers from the root and samples of dentin adjacent to the pulp were also analyzed in some cases. Lower concentrations of strontium were usually found in the outer than in the inner portions of the enamel. Dentin sometimes contained more and sometimes slightly less strontium than enamel. In 2 groups of teeth, which were studied in detail, the outermost layer of the root and the dentin adjacent to the pulp contained lower concentrations of strontium than neighboring dentin. There was a marked variation in the level of strontium in teeth from different areas. In enamel, the least amount, 15 ppm, was found in teeth from Lewiston, Maine, and the greatest amount, 350 ppm, in teeth from Big Lake, Texas. Teeth from a primitive population of the Nauru Islands in the Pacific and ancient American Indian teeth (5,000 years old) contained strontium within the range found in contemporary American teeth. Strontium levels as high as 540 ppm were found in samples of dentin from Big Lake, Texas.

56. The Nature of Water on the Apatite Surface.—*Howard M. Myers, University of California, San Francisco.* Apatite crystals are able to bind quantities of water and other polar liquids in amounts which are greatly in excess of the quantity needed to form a monolayer. The volume of liquid so held is as great as the total volume of solid present. The binding of such a

large quantity of liquid does not result in any alteration of the density of the solid phase no matter what the density of the liquid bound. Polar liquids do not form denser films around apatite than do nonpolar liquids, although nonpolar molecules are not as tenaciously held. Since a tenfold increase in molecular volume of the liquid did not alter the specific gravity of the apatite, the penetration of pores of the absorbent is not involved in the retention of liquid by apatite. Starting with a wet paste of apatite having a solid/liquid of 1.12, evaporation of 80 per cent of the water can be accomplished in an atmosphere of 90 per cent humidity. The remaining 20 per cent is removable by evaporation over $CaCl^2$ at room temperature. This portion of water, approximately 160 mg. per gram of apatite, constitutes enough water to cover the surface of apatite 9 times.

57. The Carbonate Content of Bones and Teeth.—*Duncan McConnell, Ohio State University, Columbus.* Arguments that the CO_2 is not contained within the inorganic (mineral) phase of tooth and bone have been predicated on the spurious assumption that the minerals, francolite and dahllite, do not contain CO_3 groups as integral portions of their structures. Recent crystal chemical investigations of a silicate mineral, seawtite, indicate that the same type of inverse relationship exists between CO_3 and SiO_4 groups as had been postulated for CO_3 and PO_4 groups in francolite. Inasmuch as the microcrystallinity of dentin does not facilitate direct investigation by crystallographic methods, useful analogies with mineral carbonate apatites cannot be disregarded on the basis of ambiguous and noncritical measurements of diverse sorts. Demonstration of the existence of carbonate silicates with variable ratios of CO_3 to SiO_4 has extremely important implications with respect to the variable ratios of CO_3 to PO_4 in animal hard tissues.

58. Effect of Fluoride Incorporation and Acid Treatment on Enamel Carbonate.—*Marguerite F. Little, Janet Rowley, and Finn Brudevold, Eastman Dental Dispensary, Rochester, N. Y.* Two aspects of an investigation of the carbonate component of human enamel are reported: the effect of naturally occurring F^- in the water supply on the CO_2 content of teeth and the effect of various agents on the carbonate component of ground enamel. Samples ground from the intact enamel of extracted teeth of persons of known age from areas with 2 and 5 ppm fluoride in the water supply were analyzed for CO_2 and F^- content. Results show that the carbonate component in enamel from high fluoride areas has an uneven distribution (less in the surface than in the subsurface) somewhat similar to that reported previously for enamel from low fluoride areas. However, mottled enamel contained more CO_2 at the surface than was found in the same age groups from a low fluoride area. In contrast to teeth from low fluoride areas there was no marked loss of the surface carbonate with increasing age in fluoridized teeth. Pooled enamel from teeth of a low fluoride area was used for studying the effect of H_2O, various acid buffers, fluoride and fluoridated acid buffers. The CO_2 component in enamel of the particle size used (10-23 m/g) is preferentially removed to varying degrees with all the agents tested.

VI. Saliva. March 20, Afternoon, Section C.

59. A Microscopic Study of the Saliva Sediment.—*S. W. Gilkerson, H. F. Brown, and G. H. Rovelstad, Dental Research Laboratory, USNTC, Bainbridge, Md.* The phase contrast microscopic examination of the sediment of

saliva in a study of dental caries-free young adults has revealed a definite cytology. Several distinct cell types, other than epithelial cells which have been described as a part of the salivary corpuscle system, have been observed with regularity. These cells have been noted to have diameters ranging from 10 to 18 microns when observed in the living state.

60. Chemical Fractionation of Human Saliva.—*Morris M. Matt and Carol U. Grimes, Western Reserve University, Cleveland.* Techniques have been devised to extract salivary mucin, to split the salivary mucin into protein and carbohydrate fractions, and to study the acid mucopolysaccharide in saliva. Forty-five fasting samples of gauze-filtered saliva were acidified with acetic acid, the precipitate washed with water, redissolved in 0.5 N sodium hydroxide, and reprecipitated with acetic acid. The resulting precipitate was washed with water, alcohol, ether, dried and weighed. The salivary mucin precipitate ranged from 51 mg. per cent to 2,068 mg. per cent. This precipitate was dissolved in 3-4 ml. of 0.5 N sodium hydroxide, at 37° C., and room temperature, each for 3 days. The solution was acidified with trichloracetic acid and the precipitate (protein fraction) washed with water, alcohol, ether, dried and weighed. Protein fraction ranged from 2 to 83 mg. per cent of saliva. The product gives positive biuret and negative Molisch reaction. To supernatant, equal volume 95 per cent ethanol was added, the precipitate washed with ether, dried and weighed. The product gives negative biuret and positive Molisch reaction. The carbohydrate portion of the mucin, ranged from 2 to 65 mg. per cent. To precipitate out acid mucopolysaccharide, an aliquot of sample extracted with 0.5 N sodium hydroxide for 4 days in cold, neutralized extracts treated with 2 volumes 95 per cent ethanol. Yields of this fraction were 10 to 100 mg. per cent. Alcohol concentration of supernatant was increased to 84 per cent and a second precipitate (carbohydrate in nature) obtained. Yields of precipitate II ranged from 7 to 250 mg. per cent.

61. Electrochemistry of Saliva.—*Joel Wisotzky, Division of Dental Medicine of the Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.* These studies cover the following electrochemical approaches applied to saliva and saliva-sugar systems in order to gain insight about the potential energy existing in sugared salivary systems which might be available as energy for initiating caries. The polarographic technic was utilized in an attempt to ascertain semiquantitatively the oxygen tension fall and simultaneous build up of reducing particles in a small area of the settling fraction in standing saliva. The results tend to indicate that there is a build up of negative particles oxidizable at a platinum electrode as the oxygen tension falls. Oxidation-reduction studies of saliva-sugar systems indicate that a large potential energy may exist, which if properly coupled, could provide energy for the initiation of caries. Also indicated in this series, is the electrical nonhomogenicity of saliva and the sensitivity of the electrode potential to agitation. Potentiometric titration of heavy metallic ions such as Au^{+++}, Pt^{++++}, Pd^{++}, and Cu^{++} by saliva indicates a rapid reduction of the metals by saliva. Such reductions show some evidence of proceeding in step-wise fashion.

62. Electrophoresis of Saliva.—*J. H. Geller and G. H. Rovelstad, Dental Research Laboratory, USNTC, Bainbridge, Md.* Samples of whole saliva, parotid saliva, and extra-parotid saliva were collected by paraffin stimulation. These samples were dialyzed against distilled water and then against veronal buffer

(0.1 ionic strength, pH 8.6). After concentration the sample was analyzed. Six proteins were identified in the saliva samples and isoelectric points and mobilities at pH 8.6 determined. The results are presented below.

SALIVA COMPONENT	MOBILITY	SALIVA SAMPLE			ISOELECTRIC POINT (pH)
		WHOLE	PAROTID	EXTRA-PAROTID	
Mucoid (sialo)	-8.6	x	x		2.5
Albumin	-6.0	x	x	x	4.8
α-Globulin	-4.8	x		x	5.0
β-Globulin	-3.4	x	x	x	5.5
γ-Globulin	-1.7		x	x	6.0
Lysozyme	+2.4	x		x	10.8

The β-globulin (probably amylase) was present in greatest concentration in all fractions (an average 0.060 per cent in whole and extra-parotid and an average 0.033 per cent in parotid). The nonparotid samples were similar, averaging 0.021 per cent for mucoid and 0.027 per cent for albumin. Parotid samples averaged 0.022 per cent γ-globulin and 0.025 per cent lysozyme.

63. Immunochemical Studies of Saliva.—*Solon A. Ellison and Paul A. Mashimo, College of Physicians and Surgeons, Columbia University, New York.* In order to study the relation between the composition of saliva, the physiology of its secretion, and oral disease, it is necessary to detect and measure individual macromolecular components. Immunochemical technics were applied to the study of saliva. Antisera were prepared in rabbits employing alum-precipitated saliva as antigen and these were examined by a gel diffusion technic. The antisera were tested simultaneously against a number of antigens among which were whole and fractionated saliva, serum, and purified serum proteins, and amylase. The presence of at least 4 unaltered serum proteins in saliva was ascertained. The occurrence of β-globulin and γ-globulin was inferred from the finding that antibodies which reacted with these proteins were present in the antisera. In addition, at least 4 specific and distinct salivary proteins were detected. Further applications of these methods are in elucidating salivary composition, in qualitative studies of specific components, and in their use as a guide in attempting fractionation of saliva.

64. Determination of Metallic Elements in Parotid Secretion.—*Thomas B. Weber, Virginia J. Lux and Harold R. Englander, USNTC, Dental Research Facility, Great Lakes, Ill.* Collections of parotid secretion from 43 caries-rampant males, aged 17 to 21 years, were made from the right Stenson's duct by means of a parotid cup. The parotid was stimulated by having each patient chew gum for 5 minutes. Aliquots of each sample or of pooled samples were analyzed with a DU flame spectrophotometer. All metallic elements reported as occurring in human fluids or tissues were studied except those in which it was not possible to eliminate interferences or in which the ultimate limit of detection was beyond the range of the instrument. Of those listed as occurring in relatively high concentration in fluids and tissues the following were studied: Fe, Cu, Mn, Co, Ni, Cr, Sn, Ti, Rb, Li, B, Ba, and Sr. Ag and V were tested among those listed as rarely present. The following values were found for the major metals: Ca 4.5 (S.D. $\pm$ 1.4), Mg 5.7 (S.D. $\pm$ 3.3), Na 48.4 (S.D. $\pm$ 31.6), and K 58.6 (S.D. 24.5) mg. per cent.

65. Comparative Electrolytic Concentrations of Submaxillary and Parotid Salivas.—*Basil L. Henriques and Howard H. Chauncey, Tufts University School of Dental Medicine, Boston.* Saliva samples were collected from

15 subjects in a fasting state. Curby cups were used to collect parotid saliva while a modification of the Schneyer appliance was employed to collect the submaxillary secretion. The stimulus, 3 grams of sweetened gum base, was renewed every 5 minutes during the collection period. The inorganic components measured were: sodium, potassium, calcium, bicarbonate, chloride, and phosphate; pH and flow rate were routinely recorded. All collections were made under a layer of mineral oil.

COMPONENTS	SUBMAXILLARY SALIVA*		PAROTID SALIVA*	
	RANGE	MEAN	RANGE	MEAN
Flow rate ml./min./gland pr.	0.18- 1.92	1.12	0.16- 2.10	1.07
pH	7.07- 7.65	7.38	7.12- 7.82	7.45
Sodium	10.2 -53.4	25.7	5.7 -79.0	31.6
Potassium	10.0 -17.3	13.5	12.8 -24.5	18.0
Calcium	3.3 - 6.3	4.5	1.2 - 2.4	1.7
Bicarbonate	3.4 -22.2	11.7	4.1 -37.1	19.2
Chloride	10.0 -42.4	23.6	10.8 -39.4	22.5
Phosphate	0.4 -10.5	3.9	8.0 -11.3	6.5

*Electrolyte values are in mEq./L.

Comparison of the respective measurements shows no apparent difference in flow rate, pH, and chloride between the submaxillary and parotid secretions. However, the calcium content of the submaxillary secretion was considerably greater than the parotid calcium values. On the other hand, the concentrations of sodium, potassium, bicarbonate, and phosphate were greater in the parotid secretion.

66. MECHANISMS GOVERNING THE SECRETION OF ELECTROLYTES IN PAROTID SALIVA.—*Howard H. Chauncey and Robert L. Degler, Tufts University School of Dental Medicine, Boston.* Among the constituents and properties of saliva reputed to be influenced by the rate of secretion are sodium, bicarbonate, chloride, pH, and buffer capacity. A previously reported study confirmed the positive relationship between flow rate and the sodium, bicarbonate, and pH values. In addition, a positive correlation was observed between the rate of flow and the calcium titer. However, the potassium titer was observed to be inversely related to the rate of secretion, while phosphorus showed no significant correlation with the secretion rate. An investigation of the physiologic processes which govern the secretion of electrolytes by the parotid gland indicated that the following mechanisms might be responsible for these relationships. (1) The carbon dioxide and water, produced by normal glandular metabolism, are converted into carbonic acid which ionizes into a hydrogen ion and a bicarbonate ion. The hydrogen ions so formed are transferred to the blood or extravascular fluid while sodium ions from the blood are transferred to the acinar cells of the gland. The sodium ions become associated with the bicarbonate ions and are secreted in the saliva. (2) Sodium and chloride ions are secreted by the acinar cells at concentrations isotonic with blood and are withdrawn by a process of reabsorption which is dependent on the rate of secretion. The combination of these processes could account for the changes in buffer capacity, pH, bicarbonate, sodium, and chloride observed with increased secretion rates.

67. FLOW RATE AND LACTATE FROM THE CONTINUOUSLY STIMULATED HUMAN PAROTID.—*Irving L. Shklair, Harold R. Englander, Naval Dental Research Facility, Great Lakes, Ill., and Howard H. Chauncey, Tufts University School of*

Dental Medicine, Boston. Four successive samples of parotid saliva were collected for 5 minutes from each of 36 male naval recruits (average age 18 years) by means of stimulation with 2 sticks of gum. The sampling technic was as follows: (1) the initial 5-minute collection was made, (2) the second sample was collected after 30 minutes (subject chewed 2 fresh sticks of gum every 5 minutes during the interval between collections), (3) the third sample was collected 60 minutes after the start of the experiment (continuous chewing and changing), (4) the subject was given a 15-minute rest period, and (5) a fourth 5-minute sample was obtained immediately after the 15-minute recovery period. The volume of parotid saliva collected at each time interval was measured and the lactic acid concentration was determined for the 5-minute volume. The mean flow rates per 5 minutes at zero time, 30 minutes, 60 minutes, and after a 15-minute rest period were 4.2 ml., 3.4 ml., 3.3 ml., and 4.5 ml. of parotid saliva. The mean lactic acid concentrations were, respectively, 19.5, 24.3, 23.7, and 22.5 meg. per ml. of parotid saliva. It appeared that the rate of secretion tended to diminish after continuous stimulation. This fatigue effect on the gland occurred concomitantly with the accumulation of lactic acid in the parenchyma and the excretion of increasing amounts of latic acid. However, the gland was able to recover after a brief rest period.

68. The Reducing Power of Human Salivary Secretions.—*Ward Pigman and William L. Hawkins, University of Alabama, Medical Center, Birmingham.* The reducing power of whole human salivas and of parotid, submaxillary, sublingual, and mucosal secretions have been measured by the Folin-Malmros method and reported as apparent glucose. Generally, the parotid and submaxillary secretions have less reducing power than the sublingual and mucosal secretions. The only noticeable effect of stimulation was observed for the parotid gland, which showed a decreased reducing power upon stimulation. In agreement with the earlier results of Lundquist, no evidence was found for the presence of any free sugars, although several different methods were tried. The various methods indicate a maximum possible concentration of glucose of about 0.5 mg. per 100 c.c. The reducing substances of the salivary secretions seem to be a heterogeneous mixture of various carbohydrates, proteins, glycoproteins, and some small molecular weight materials.

69. Amino Acid Decarboxylases in the Sediment From Incubated Saliva.—*N. Gochman, R. Q. Blackwell, and L. S. Fosdick, Northwestern University Dental School, Chicago, Ill.* Salivary bacteria, cultivated under varying conditions, were tested for the presence of nonoxidative amino acid decarboxylases. Nonincubated saliva as well as acetone-extracts, obtained from glucose-casein digest cultures, showed no detectable activity. Aerobic incubation of whole saliva, in the presence of casein hydrolysate for several hours, yielded a sediment which exhibited decarboxylase activity. The presence of glucose inhibited the development of the decarboxylases. The washed sediment was tested on 17 amino acids used as substrates. Arginine, ornithine, glutamic acid, histidine, and lysine were consistently decarboxylated, with no measurable activity on the other amino acids. Determinations were performed using a manometric estimation of CO_2 evolution under N_2 atmosphere. The pH optimum was 6.5 for each of the 5 amino acids, considerably more alkaline than that reported for other bacterial amino acid decarboxylases. A study was made of the relative distribution and activity of the decarboxylases in saliva obtained from normal and periodontal patients. The diseased patients showed appreciably greater total decarboxylase activity than the normals. However,

the distribution of amino acid decarboxylases in all saliva samples remained relatively constant, with ornithine accounting for about 50 per cent of the total CO_2 evolved.

70. The Production of Ammonia and Volatile Amines in Saliva.—*L. S. Fosdick, R. Meyer, Jr., and R. Q. Blackwell, Northwestern University Dental School, Chicago, Ill.* Saliva was collected by paraffin stimulation from subjects selected clinically as being periodontally normal, or as having suffered severe periodontal bone loss. A measured amount of saliva was incubated for 4 hours with added casein and was then centrifuged and divided into 2 portions each of which was made alkaline and distilled in a micro-Kjeldahl apparatus. The volatile alkaline substances were collected in boric acid and titrated with 0.02 N HCl. After the sample was titrated it was transferred to a volumetric flask, nesslerized, and the amount of ammonia estimated by means of a Coleman spectrophotometer. The results were expressed as microequivalents per 100 ml. saliva. It was found that the saliva from patients suffering with periodontal disease usually formed a larger amount of volatile amines than the saliva from periodontally normal persons.

VII. Experimental Caries. March 21, Forenoon, Section A.

71. Survey of Egyptian Rodents for Caries Production.—*Maurice A. Mazzarella and Vincent J. Berzinskas, Naval Medical Research Unit No. 3, Cairo, Egypt.* Four different types of rodents from the Cairo area were placed on cariogenic diets for 77 days. They were the Nile grass rat *(Arvicanthus niloticus)*, the kangaroo rat *(Jaculus jaculus jaculus)*, the gerbil *(Gerbillus gerbillus gerbillus)*, and the hedgehog *(Hemiechinus auritus aegyptius)*. The animals numbered 36 of each type, and were equally divided among 3 different diets. They were caged individually with only desert sand for bedding. Bacterial plaques were noted on the buccal surface of hedgehog molars as early as 30 experimental days. The other types of animals did not present such dramatic findings even at the end of the feeding period. Two hedgehogs showed what were considered to be carious lesions in fissures of molar teeth. No gross lesions could be demonstrated under the heavy dental plaques seen on the molars of all hedgehogs. The gerbils and the kangaroo rats showed accumulation of food debris around the necks of the teeth and heavy staining in the pits and fissures. One gerbil showed heavy deposition of a bright orange-colored calculus. The grass rat presented the cleanest teeth at the end of the experimental period.

72. Caries Initiation and Promotion Factors in the Rat.—*Willard G. Clements, Edward C. Hilger, Jr., Nicholas King, John F. Stasik, Sara D. Goodlin, and Gerald J. Cox, School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* Littermate rats from 61 litters, weaned at 21 days, were placed, respectively, on (I) a ⅔ sucrose ration for 8 weeks and (II) the Hoppert-Webber-Canniff coarse rice ration for 2 weeks and then transferred to the ⅔ sucrose ration for 6 weeks. Thus, the first and second molar teeth of the second group were exposed to initiation of carious lesions before the third molar teeth were erupted. In Group I, 23 of 201 rats showed 50 carious lesions with only 1 lesion in a lower third molar. In Group II, 134 of 190 rats had 502 carious lesions with 73 being in the lower third molars. This indicates that the presence of active carious lesions, with their associated microorganisms, possibly contribute to the initiation of the carious process. However, in 8 animals of Group II, carious lesions appeared only in the lower third molars, indicating that maturation of the enamel on the Hoppert-Webber-Canniff ration was relatively defective.

73. THE SYSTEMIC ETIOLOGICAL FACTORS OF CARIES IN WHITE RATS.—*Ralph R. Steinman, C. Gordon Hewes, and Mervin G. Hardinge, College of Medical Evangelists, School of Dentistry, Loma Linda, Calif.* To determine if sugar has a systemic effect in the production of caries, in addition to its local effect, the following experiments were conducted: (1) A diet composed of typical American foods in average amounts, minus desserts, was fed to 21 rats from weaning for 13 weeks. Ten animals received injections of a 20 per cent mixture of equal parts of glucose and fructose intraperitoneally, t.i.d., in 1 gram to 1,000 grams body weight dosage, given from the twenty-first day through the thirteenth week. Molars were examined by the method of Shaw. Those receiving injections scored 10.9, control littermates, 3.18 (t value, 2.79). (2) Adding 16 mg. vitamin B_6 per kilogram to rations, composed of typical American foods including desserts in average amounts, reduced caries. Animals were sacrificed after 13 weeks on diet. The score on diet alone was 8.8, for littermates on diet plus B_6, 4.0 (t value, 2.2). (3) In 0.5 ml. dosage, trypan blue (2 per cent) was injected intraperitoneally into white rats, once a week for 3 weeks. Littermates were divided into 2 groups at weaning; one was placed on Purina, the other on high sucrose diet. After 12 weeks, the teeth of animals on Purina were visibly bluer than those on high sucrose diet. Other animals were injected on the thirty-fifth day with 0.5 ml. of 4 per cent trypan blue and sacrificed after various periods of time. The tooth structure became visibly discolored within 10 minutes, and became bluish in 24 hours. These studies tend to indicate that a disturbance in metabolism is an important factor in the dental caries problem.

74. THE INFLUENCE OF NONGENETIC FACTORS IN TRANSMISSION OF HAMSTER CARIES.—*Paul H. Keyes, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* Young, newly weaned hamsters, furnished by the Animal Production Station of the National Institutes of Health, generally show a high degree of resistance to dental caries when placed on the cariogenic diet now used. High, but somewhat more variable, resistance is found in animals born, raised, and weaned in our laboratory from pregnant females furnished by the Animal Production Station. First generation animals from parents exposed to our laboratory conditions and test diets generally become quite susceptible although the parents have been resistant. A critical test for demonstrating possible transmissible factors was conducted by dividing 37 piebald hamsters into 2 groups, by placing 19 animals in cages with young albino hamsters of known susceptibility, and by keeping 18 animals by themselves. The diet, water, type of cage, and number of animals per cage (approximately 4) were equalized for both groups. At the end of the 56-day experimental period only isolated animals failed to show a high degree of caries activity. In the isolated group, 42 per cent of teeth had minor lesions; scores ranged between 0 and 15 (average 3.5). Only 1 animal had lost the equivalent of 2 molar cusps. In contrast, all animals that were exposed to susceptible hamsters had caries; 94.3 per cent of teeth were affected; scores between 6 and 216 averaged 75; 27.6 per cent of teeth were half or more destroyed.

75. EFFECT OF VARIOUS METAL SALTS OF ETHYLENEDIAMINE TETRAACETIC ACID ON DENTAL CARIES IN THE RAT.—*L. C. Hendershot and Janet Forsaith, The Biochemical Research Laboratory, The Dow Chemical Company, Midland, Mich.* Weanling male rats (Dow's colony of the Wistar strain) were placed on a cariogenic diet (50 per cent sucrose—50 per cent Purina Laboratory Chow) containing 5 dose levels (1.0, 0.5, 0.25, 0.125, and 0.063 per cent) of the following 9 salts of EDTA: di-Na^+, Mg^{++}, Ca^{++}, Mn^{++}, Fe^{++}, Co^{++}, Zn^{++}, Ni^{++}, and Cu^{++} (presented here in order of increasing stability as chelates). Caries scores were appreciably greater than controls (3 to 4 fold) in animals receiving the di-Na^+,

Mg^{++}, and Co^{++} salts and appreciably less (¼ to $\frac{1}{10}$ of control scores) in animals receiving the Zn^{++} and Ni^{++} salts. Less outstanding differences in the caries scores were detected in animals fed the Ca^{++}, Mn^{++}, Fe^{++}, and Cu^{++} salts. The significance of these differences will be discussed in light of the toxicity and chelate stability of the compounds administered.

76. Changes Induced in Cariogenicity of Diets by Interchanging Salt Mixtures.—*John Haldi, Winfrey Wynn, and James H. Shaw, Emory University, Emory University, Ga., and Reidar F. Sognnaes, Harvard School of Dental Medicine, Boston.* In 3 different experiments in the Emory laboratories, substitution of the Emory salt mixture for the Harvard salt mixture in the Harvard diet reduced the cariogenicity of the Harvard diet. Conversely, replacement of the Emory salt mixture by the Harvard salt mixture in the Emory diet increased the cariogenicity of the Emory diet. The effects obtained in the Harvard laboratories by interchanging the salt mixtures of the 2 diets were not as striking nor as consistent as in the Emory laboratories. In two experiments, the animals that received the Emory salt mixture prepared by the Emory procedure had appreciably less tooth decay than those fed the Harvard salt mixture. The differences, however, were less than those observed in the Emory laboratories. In a third experiment, the cariogenicity of the diets was not affected by the interchange of salt mixtures. Another salt mixture was also prepared in the Harvard laboratories from dry reagent chemicals in proportions that gave the same ratios of nutritionally essential anions and cations as the Emory salt mixture. This salt mixture was not effective in reducing the cariogenicity of the Harvard diet. These experiments suggest that the mechanism operative in producing dental caries by the 2 diets is of a delicate nature and emphasizes the complexity of the dental caries problem.

77. Studies on the Cariogenicity of the Emory and Harvard Diets.—*Winfrey Wynn, John Haldi, M. L. Law, and K. D. Bentley, Emory University, Emory University, Ga.* The following experiments were conducted to determine what factor or factors in 2 high sucrose diets with marked differences in cariogenicity may be responsible for the difference. All experiments were conducted using desalivated littermate rats of the Wistar strain. (1) The Harvard vitamin mixture was replaced by the Emory vitamin mixture in the Harvard diet and vice versa in the Emory diet. (2) Five per cent dried brewers' yeast was added to the Harvard diet. (3) Four per cent yeast and liver extract was added to the Harvard diet. (4) One mg. and 10 mg. of pyridoxine hydrochloride per 100 Gm. was added to the Harvard diet. (5) The Harvard and Emory diets were ground to the same fineness (60 mesh). (6) The concentration of magnesium in the Emory and Harvard diets was varied. (7) Manganese was added to the Harvard diet in amount of 50, 150, and 500 ppm. (8) The Na/K ratio of the Emory diet was changed to approximate that of the Harvard. (9) The iron concentration in the Emory diet was varied by (a) adding no iron, (b) adding ½ the regular amount, and (c) adding twice the regular amount. Variation of these factors did not affect the relative cariogenicity of the Harvard and Emory diets. It has been previously reported that variation of the Ca/P brought about by the addition of Ca or P to the Emory diet resulted in a marked reduction in the cariogenicity of this diet.

78. Effect of Trace Element Sea Salt Mixture on Rat Caries.—*Erling Johansen, William Strain, and Peter Barnard, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* Individual trace elements incorporated into the diet or administered in the drinking water of experimental animals maintained on a cariogenic diet may inhibit, accelerate, or be without apparent

effect on the process of dental caries. Since sea salt contains a mixture of many elements, it was of interest to determine its effect on the caries process in rats maintained on a highly cariogenic diet. The following 3 groups of 19-day old animals were set up: I, 580 diet (66 per cent sucrose, 32 per cent skimmed milk, 2 per cent liver powder); II, 580 plus 4 per cent sea salt mixture; III, 580 plus 6 per cent sea salt mixture. For correlation of caries experience of the individual molars with trace element composition of the tooth, animals were sacrificed at various intervals throughout the experimental period of 75 days. Final total caries scores (after 75 days) were obtained on 12 ♂ and 16 ♀ in Group I; 12 ♂ and 16 ♀ in Group II, and 7 ♂ and 9 ♀ in Group III. The scores and standard deviations were as follows: I, 40 ± 7.7; II, 48 ± 7.6; III, 63 ± 10.6. The apparent increase in mean caries scores with higher trace element composition of the diet was not of statistical significance.

79. Effect of Platinic Chloride on Experimental Dental Caries in Rodents.—*John W. Hein, Gertrude A. Quigley, and Hans Marcussen, Division of Dental Medicine, Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.* Hamsters, mice, and rats obtained from commercial sources are generally more caries resistant than those raised under special laboratory conditions, thus, a demonstration that their caries susceptibility could be increased by a low level of platinic ion in the drinking water might prove a useful technic in caries research. Control and experimental groups consisting of 15 males and 15 females of each species were placed at weaning on a caries-producing diet for 95 days. During this period, the control animals received distilled water, the experimental animals received a 1 mEq. per liter platinic ion solution made from platinic chloride and neutralized to pH 5.5 with NaOH. At the end of 95 days only the rat groups showed sufficient dental caries to warrant sacrifice. Mean caries scores for the control and experimental rats were 8.75 and 13.96, respectively. This difference was statistically significant. All lesions were of the occlusal type and the platinic group showed an increase in numbers of carious teeth, of carious areas, and of large size cavities. The influence was exerted on both the maxillary and mandibular teeth. It appears that platinic ion stimulates the initiation and progression of dental decay in caries-susceptible rats, a finding formerly reported for Syrian hamsters.

80. The Effect of 2 Per Cent $CaHPO_4$ Dietary Supplement on Experimental Dental Caries in the Rat.—*Peter Barnard and Erling Johansen, School of Medicine and Dentistry, Rochester, N. Y.* Caries development may represent an unfavorable ionic balance between the tooth surface and the local oral environment. An attempt was made to favorably alter that balance by the addition of $CaHPO_4$ to the diet. Three diets of different caries-producing potential were used in this study of 131 rats. For each diet, 25-day-old male and female animals were set up into control and experimental groups, the experimental groups receiving the respective diet with 2 per cent $CaHPO_4$ incorporated. The composition of the diets, the number of animals in each group and the experimental periods were as follows: Diet I: 66% sugar, 32% skimmed milk powder, 2% liver powder; control (10 ♂, 12 ♀) and experimental (11 ♂, 13 ♀); experimental period, 85 days. Diet II: 35% sugar, 30% whole wheat flour, 30% whole milk powder, 4% alfalfa powder, 1% sodium chloride; control (12 ♂, 13 ♀) and experimental (10 ♂, 10 ♀); experimental period, 100 days. Diet III: 20% sugar, 20% starch, 20% whole wheat flour, 30% skimmed milk powder, 5% alfalfa powder, 5% sodium chloride; control (9 ♂, 12 ♀) and experimental (7 ♂, 12 ♀); experimental period, 120 days. In the severely cariogenic Diet I, the addition of 2 per cent $CaHPO_4$ was ineffective in reducing dental caries. In the moderately and relatively low cariogenic Diets II and III, the 2 per cent $CaHPO_4$

effected a statistically significant reduction in dental caries on the bases of teeth affected, areas, and scores. The caries reduction in experimental Diet III confirms previous observations by Stralfors.

81. Effect of Phosphate Supplement to Diet on Development of Hamster Caries.—*A. E. Nizel, Noreen Keating, Carol Sundstrom, and Robert S. Harris, Massachusetts Institute of Technology, Cambridge.* Eight female and 6 male weanling hamsters were maintained on Keyes cariogenic diet for 6 weeks, then on a 1:1 mixture of Purina Lab Chow and NIH special rabbit-guinea pig ration for 3 weeks. In vivo examination by binocular microscope revealed that each hamster had developed 2 or more carious molars. These hamsters were mated, 2 females producing 1 litter each and 5 producing 2 litters each. When 42 days old, these 44 weanling hamsters were divided into 2 groups with equal distribution according to littermates and sex. Group A was offered the Keyes diet and Group B was fed the Keyes diet modified by supplementation with metaphosphoric acid. Thus, Group A received 0.43 per cent P and Group B received 0.86 per cent P in the diet. After 42 days ad libitum on these diets and distilled water, they were sacrificed, and the molar caries scored by the Johansen technic. The mean caries score was 30.0 for Group A and 7.6 for Group B. The caries in littermate pairs was compared by 4 statistical approaches, all indicating that the difference was highly significant. The addition of phosphate to MIT diet No. 10 was reported to cause a marked reduction in dental caries in hamsters. The same observation has been made using a different diet and a different phosphate. The level of phosphate in the diet has an important influence on dental caries in hamsters. The dental caries was definitely more severe in the F_{1b} litters than in the F_{1a} litters. This effect does not appear to be related to the diet. The interlitter variations in caries susceptibility were much greater than the intralitter variations.

82. Preliminary Observations on the Effect of Selenium Salts on Caries Production in Rats.—*C. D. Walthall, B. W. Warner, Hugh I. Myers, and C. V. Gillooly, University of Kansas City, School of Dentistry, Kansas City, Mo.* For 20 days on each series of 4 female rats and 1 male rat, selenium solution (sodium selenite) of 1 ppm was fed with standard chow. At end of this period, each pregnant female was placed in a separate cage and fed a normal diet and selenium water. When the progeny were 20 days old, they were separated and placed 4 to a cage. A cariogenic diet and selenium water was initiated with these rats for 56 days. Controls were carried through by the same methods except for the elimination of selenium water. After 56 days, the series of experiments and controls were sacrificed, heads removed, and jaw teeth examined by explorer and dissecting microscope. The total caries appearing on all surfaces of each of the jaw teeth in a total of 116 rats were counted and tabulated. A significant difference exists between the total caries counted in the selenium experimentals and controls. The greater number of caries occurred in the control animals. No definitive conclusion will be drawn at present because of inadequate sampling material, but further investigation is warranted.

VIII. Transplantation and Repair. March 21, Forenoon, Section B.

83. Preservation and Storage of Tooth Germs.—*Robert Gerstner and Earl O. Butcher, Murry and Leonie Guggenheim Foundation Institute for Dental Research, and New York University College of Dentistry, New York.* Freezing and thawing experiments to determine the feasibility of preserving and storing tooth germs and pulp for future use (implantation, transplantation, etc.) were

performed on molar and incisor tooth germs dissected from rats of 17 to 21 days fetal age. The tissues were frozen in liquid nitrogen (–190° C.), isopentane cooled in liquid nitrogen (–120° to –150° C.), and solid carbon dioxide dissolved in ethyl alcohol (–78° C.). Freezing was accomplished by immersing tissues directly into the freezing materials or indirectly within containers of different insulating capacities. Rates of freezing were also investigated. Tissues were frozen with and without prior immersion in glycerol-saline and ethylene glycol-saline solutions of various concentrations, previously found to protect tissues from many harmful effects of low temperatures. Results were observed and evaluated by survival and growth in tissue cultures. Tooth germs survived rapid freezing when given protective pretreatment in glycerol and ethylene glycol solutions, and continued to grow in tissue cultures. Growth was at a decreased rate and did not reach the developmental levels attained with unfrozen controls. Slower freezing technics tended to accelerate growth rates and augment development. Tissues frozen without the protective pretreatments did not survive in tissue cultures.

84. Homogenous Transplantation of Hamster Second Molars From Young to Adult Animals.—*Robert B. Hoek, Emmett R. Costich, and James R. Hayward, University of Michigan, Ann Arbor.* The experimental group was composed of 7 female and 12 male hamsters of approximately 2 months in age. The lower right and left second molars were extracted from the adult animals and replaced by the developing second molars removed from 6-day-old hamsters. Two of the animals had only 1 transplant due to fracture of the other second molar during the extraction procedure. Three animals died of undetermined cause during the course of the experiment. The animals were sacrificed after 75 days. Gross examination showed that 10 of the transplants had been sloughed, 16 were hypermobile, and only 4 were firm. Histologically, 13 of the 20 specimens showed pulp necrosis. Eight teeth had vital connective tissue in the root canals. There was good periodontal membrane reattachment in 3 instances, fair in 2 instances, and poor in the remainder. No ankylosis was observed. There was evidence of sequestration in about half of the cases, but it was periapical and not intraradicular, as seen in our previously reported transplantation studies. Only 5 teeth showed resorption; but this is not unusual since resorption only takes place when there is periodontal attachment, and only 5 teeth showed any degree of reattachment.

85. Replantation of Molar Teeth in the Syrian Hamster. II.—*Emmett R. Costich, Robert B. Hoek, James R. Hayward, University of Michigan, Ann Arbor.* Sixteen male and 13 female 21-day-old hamsters were utilized. The lower right 2nd molar was replanted; however, if there was fracture of this tooth during surgery, the lower left 2nd molar was utilized. The animals were sacrificed at regular intervals between 14 days and 3 months. Gross findings revealed that 3 of the 29 teeth had been avulsed. Of the teeth remaining, half were retruded or submerged in their sockets to varying degrees and the other half were in normal occlusion. Histologic examination revealed that 4 of the replantations had been perforated during the course of surgery. The pulps of the teeth were necrotic. Of the teeth without perforations, 7 had necrotic pulps. The pulp tissue in the roots of the replants was found to be vital in most cases. In some cases where the tissue in the pulp chamber was necrotic, that in the root canals was still vital. In many instances, the tissue in the root canals resembled periodontal membrane. In 4 of the replants, the root canals were almost filled with osteodentin. The teeth of the animals sacrificed early in the experiment showed no organized arrangement of fibroblasts in the periodontal membrane. As time progressed, the fibroblasts took on a normal arrangement in relation to the root

and alveolar bone. In 4 teeth there was periapical abscess formation. Ankylosis occurred in almost half of the cases. Resorption was present to some degree in all but 1 tooth.

86. Long-Range Postoperative Evaluation of Survival of Hamster Second Molars.—*Viola D. Flanagan and Hugh I. Myers, University of Kansas City, School of Dentistry, Kansas City, Mo.* Previously, it has been shown that successful takes can be improved by diet control, and that teeth can be as successfully transplanted as replanted. These results were based on 30-day postoperative observations, hence it was not determined how long successful takes would survive in their new environment. Since it has required 2 years for completion of the long-range study, these data are based on the dietary procedures used at that time and do not incorporate the improvements that have since been developed. Twenty-eight replanted teeth with soft diet and sacrificed 30 days postoperatively showed 25 per cent good takes; 28 transplanted teeth with hard diet, sacrificed 30 days postoperatively, 36 per cent good takes; 34 replanted teeth with hard diet, sacrificed 30 days postoperatively, 57 per cent good takes; 28 transplanted teeth with hard diet, sacrificed 60 days postoperatively, 57 per cent good takes; and 33 transplanted teeth with hard diet and sacrificed 18 months postoperatively showed 21 per cent good takes. Transplants and replants give comparable figures with the same diet. Hard diet shows approximately twice as many good takes as soft diet, hence the improvement at 60 days postoperatively could be attributed to the diet. The decrease in number of expected good takes at 18 months would indicate some of the poorer good takes did not persist however, it also indicated the better good takes did persist.

87. Blood Vessels and Hormones in Castrates Bearing Transplanted Tooth Germs.—*Harold S. Fleming, Yale University School of Medicine, New Haven, Conn.* Male and female guinea pig castrates received homotransplants of tooth germs to the eyes and brains. Following this, stilbestrol was given to female hosts and testosterone to male hosts in 1 mg. intramuscular injections 3 times weekly. At periodic intervals, between 3 and 25 injections, host animals were killed and the transplants recovered. The fate of in vivo transplanted tooth germs depends upon the biologic activity of small blood vessels within the dental pulp and in surrounding connective tissue. In guinea pigs that are castrated, vascularity to transplants is maintained for longer periods than in intact hosts. When these substitutive hormones are administered, vascularity to transplants is considerably increased. This augmented vascularity, however, does not aid the homeostasis of these explants for, as these hormonal injections are continued, small blood vessels within the dental pulp increase in size and in number. Ultimately these vessels become so aggressive that odontoblasts and other pulpal cells are pushed out of place by their activity. There is also a marked increase in red blood cells within the vessels in the connective tissue surrounding these transplanted tooth germs. Thus, transplants are unable to continue growth and development.

88. Reactions of Various Rat Epithelia to Local Injections of Growth Hormone.—*S. S. Stahl, R. Gerstner, and O. Joly, Murry and Leonie Guggenheim Foundation, Institute for Dental Research and New York University College of Dentistry, New York.* Forty-nine Long-Evans strain rats (40 males and 9 females) were divided into experimental and control groups which received local subcutaneous injections into either the oral mucosa at the mucobuccal fold or near the mucocutaneous junction of the anus or vagina. Hormone injections consisted of 0.5 mg. of somatotrophic hormone buffered to a pH of about 10 in 0.1 c.c. of distilled water, given 3 times weekly for a total of 6 weeks. The control injections consisted of 0.1 c.c. of distilled water, buffered to a pH of about

10, given in a similar manner as in the experimental group. Eleven additional Long-Evans strain rats, 8 males and 3 females, were kept as untreated controls. At the end of the experimental period, all animals were sacrificed, the injection site dissected out and prepared for histologic and tissue culture study. Epithelia of different locations responded in varying degrees to hormone treatment. Anal mucosa showed some increase in the size of the epithelial layer but appeared to be least affected of all the epithelia studied. Oral epithelium responded with an increase in keratinization and marked acanthosis. A great number of binucleate cells were observed in the stratum intermedium. These nuclei stained distinctly with prominent nucleoli and appeared to be joined. Some increase in individual cell size of this layer was also noted. Treated vaginal mucosa showed a marked epithelial hyperplasia; however, binucleate cells were not observed. Increased growth of epithelia was not observed in any of the control specimens.

89. Effect of Age on Acute Inflammation.—*Bernard K. Forscher and Helene C. Cecil, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* Tissue from 12-month-old Sprague-Dawley rats was analyzed before and at specific time intervals after the generation of a standard experimental acute inflammation in the antemolar region of the palate. The concentration of each tissue component was plotted against time during the inflammatory response up to 14 days. On comparing these curves to curves from 3-month-old rats, treated in an identical manner, there was no difference found for total introgen, tyrosine, or RNA. The height of the glycogen maximum was increased and the heights of the maxima for DNA and acid-soluble organic phosphorus were decreased. The terminal portions of the curves for glycogen, water, hydroxyproline, hexosamine, and nonprotein nitrogen were altered.

90. Experimental Production of Oral Ulcerations in the Rat.—*S. Jerome Zackin and Paul Goldhaber, Harvard School of Dental Medicine, Boston.* Extensive gingival and labial mucosal ulcers progressing to "gangrenous stomatitis" were reported as produced in cortisone-injected rats following repeated clipping of the lower incisors to the gingival margin (referred to below as "cut-down incisors"). An attempt was made to reproduce these findings. Thirty-two female albino rats were divided into 4 groups: normal controls, cortisone-injected (Cortone acetate, Merck, 2 mg./day), "cut-down incisors," and "cut-down incisors" plus cortisone-injected. After 30 days, none of the animals with intact incisors exhibited ulcerations grossly or histologically. The groups whose incisors were cut down showed ulcerations regardless of cortisone treatment. Ulcerations of the labial mucosa, gingiva, or tongue were present in all but 1 animal of each group, appearing somewhat more extensive in the cortisone-injected animals. Microscopic examination revealed ulcers extending diffusely into the submucosa. Acute and chronic inflammatory cells were present, with polymorphonuclear leukocytes predominating. No marked hemorrhage, vascularity, or edema was present.

91. Effects of Beta-Aminopropionitrile Upon Wound Healing.—*George A. Krikos and J. L. Orbison, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Beta-aminopropionitrile has been shown to have pronounced effects upon mesodermal tissues. In this study, wound healing was used as a tool to determine the effects of this compound on the formation of fibrous connective tissue in the absence of any significant mechanical stress. Weanling and adult male rats were divided into groups of 8 rats each. All animals were pair-fed and received Purina Fox Chow; 0.4 per cent beta-aminopropionitrile fumarate was added to the diet of the experimental rats. On the ninth experimental day an excision wound, 1 cm. in diameter, was made in the

right posterolateral chest wall. Photographs of the wounds were taken every third day. Control and experimental groups of weanling rats were sacrificed on the third day and ninth day after wounding, while similar groups of adult rats were sacrificed on the third and twelfth day. The wounds with their surrounding tissues were excised, fixed in formalin, and stained with hematoxylin and eosin. In the weanling experimental rats, wound contraction was markedly decreased. The granulation tissue was thinner and showed numerous extravasated red blood cells and foci of an eosinophilic material containing fine, disoriented fibrils. The number of collagen fibers and fibroblasts per unit area was decreased and these cells were more spindle shaped than in the controls. In the cut edge of the panniculus carnosus, the fibroblastic proliferation was more intense in the experimental rats. Epithelization of the experimental wounds was not affected. In the adult experimental rats, contraction and epithelization were not affected and changes in the granulation tissue were relatively slight.

92. Phenergan Hydrochloride and Extraction Wound Healing in the Hamster.—*Richard Miyahara, Hugh I. Myers, and Viola D. Flanagan, University of Kansas City, School of Dentistry, Kansas City, Mo.* Thirty-day-old hamsters had bilateral mandibular second molar extractions. The experimental animals were given 0.03 c.c. Phenergan hydrochloride 1 day preoperatively, the day of surgery, and 1 day postoperatively. The control animals received none. Both experimental and control animals were given a normal diet of Purina Chow and green vegetables. The animals were sacrificed 3 days, 1 week, and 3 weeks after the surgery. The mandibles were removed and histologic sections prepared. In the 3-day experimentals, the alveolar socket showed fibroblasts and collagenous fibers next to the bone, then a zone of early granulation tissue and, in the center, some red blood cells. The impression was gathered that the 3-day control animals showed more collagenous fibers in the extraction socket. In the 1-week group of animals, the alveolar sockets were filled with fibrous connective tissue. The 3-week animals showed the extraction socket filled with heavy spicule bone. No consistent difference was seen between the experimental and control animals in the 1- and 3-week groups. Although these findings do not necessarily contradict the previously reported clinical beneficial effects of this drug, including reduction of swelling, it does seem to indicate no increased cellular activity in wound healing as a result of its use.

93. In Vivo Titration of Connective Tissue Colloids.—*M. B. Engel, N. R. Joseph, H. R. Catchpole, and D. M. Laskin, University of Illinois, Colleges of Dentistry, Medicine, and Pharmacy, Chicago.* The colloidal charge density in connective tissues was studied using an electrometric method. The charge depends on the state of aggregation of the colloids, on their composition, and on the pH of the tissue. By varying the pH at the tissue surface, it is possible to obtain a curve showing the changing charge density with pH. This is equivalent to a titration curve of connective tissue, in vivo. Isotonic NaCl solutions of pH varying between 7.4 and 2.2 at ionic strength 0.15 were used. Tissues studied included cartilage, tendon, and skin (dermal layer) of rat and rabbit. The base binding at physiologic pH and the isoelectric points are given.

	NEG. CHARGE DENSITY AT PH 7.4 (EQ. PER KG. TISSUE H_2O)	ISOELECTRIC POINT
Cartilage	0.18	2.1
Skin	0.05	2.2
Tendon	0.02	2.5

As the pH is lowered the negative charge density of the tissue decreases because of the binding of hydrogen ions. Most of the decrease occurs at pH

values lower than 4, corresponding to the range of dissociation constants for the carboxylic acid groups of proteins. The negative charge density becomes zero at the isoelectric point which varies from pH 2.1 to 2.5. The low isoelectric points depend on the presence of large amounts of acid groups which combine with hydrogen ions only below pH 2.2. Sulfate groups of the mucopolysaccharides and glycoproteins of the extracellular substance fall into this category. Inorganic or organic cations combine with the colloids and, in consequence, produce modifications of the titration curves.

94. A Test of Connective Tissue "Function" in Health and Disease.—*Daniel M. Laskin, Milton B. Engel, Norman R. Joseph, and Victor E. Pollak, Colleges of Dentistry, Pharmacy, and Medicine, University of Illinois, Chicago.* The rate at which connective tissue ground substance is disaggregated and restored after injection of hyaluronidase may be an index of its initial state and its capacity for repair. From theoretical considerations, recovery rate can be related to the hydrodynamic flow of electrically charged colloids as well as to their synthesis by cells. This reaction was studied by an electrometric method involving serial measurement of liquid junction potentials in the dermis using 0.15 and 0.015 M NaCl. From the dilution potential (E_d), the colloid charge density (X) was calculated by the formula: $E_d = -12.3 + 206\ X$. After the initial dilution potential was determined, 15 TRU of testicular hyaluronidase was injected at the test site and the displaced dilution potentials (E'_d) were measured over a 4-hour period. Normal persons and patients with "collagen diseases" were compared. The following results were obtained.

	INITIAL E_d	E'_d (MAX.)	HALF RECOVERY TIME
Normal (6)	-2.0 mv	- 8.1 mv	140 min.
Lupus erythematosus (2)	-5.8 mv	-11.0 mv	none in 4 hr.
Scleroderma (3)	-8.9 mv	-11.6 mv	none in 4 hr.
Rheumatoid arthritis (1)	-9.0 mv	-12.0 mv	none in 4 hr.

After adrenal corticosteroid treatment, the patients with lupus erythematosus and scleroderma showed less negative dilution potentials, less displacement after hyaluronidase, and a more rapid half-recovery rate. Although this treatment does not completely reverse the electrometric pattern, it appears to produce a response more closely resembling the normal. In 3 patients with naturally occurring hyperfunction of the adrenal cortex, half-recovery rates were even more rapid (65 min.) than normal. Measurement of the changes in the state of the ground substance after administration of hyaluronidase appears to be a useful test for evaluating connective tissue diseases and the effects of therapy.

IX. E. H. Hatton Award Competition. March 21, Forenoon, Section C.

95. The Development of a Technic for Root Canal Experiments in Hamster Molar Teeth.—*Leslie R. Burrows, University of Kansas City, School of Dentistry, Kansas City, Mo.* The first molar teeth in 30-day hamsters were used. After Nembutal anesthesia, the animal's head was fastened in an appliance designed to hold the mouth open and immobilized. The crown of the first molar was opened with a 33½ inverted cone bur. Using a No. 3 Kerr root canal reamer, the pulp was removed to a predetermined length of 2.5 mm., No. 2 silver points were cut to the 2.5 mm. length, Kerr sealer applied to the point, and the point inserted into the root canal. Postoperatively, the animals were fed the regular diet of Purina Checker and green vegetables. Preliminary histologic examination of the teeth from animals sacrificed 3 days, 1 week, and 3 weeks

showed some periapical inflammatory reaction. Although more extensive studies are indicated before conclusions should be drawn, it was felt the inflammation was possibly less intense after 3 weeks.

96. Histologic Changes Produced by Pressure Injection of Whole Blood Into the Intermedullary Spaces of Dog Mandibles.—*P. J. Holton, Providence, R. I.* Whole autogenous blood was forcibly injected into the intermedullary spaces of 7 dog mandibles in an attempt to create a hemorrhagic cyst. One dog was sacrificed at 2 days, 2 dogs at 10 days, 2 dogs at 3 weeks, and 2 dogs at 4 weeks. Microscopic studies of the injection site of the 2-day dog revealed discrete areas of frank hemorrhage throughout which were scattered debris and acute inflammatory cells. The 10-day dogs exhibited the first signs of organization and repair in the area of the bur hole used for the injection of blood. The 3- and 4-week dogs demonstrated a progressive filling in of the bur hole areas with fibrous tissue and new (osteophytic) bone. The intermedullary hematoma formed by the injection of whole autogenous blood did not produce hemorrhagic cysts. The response was one of inflammation and repair, caused by the trauma of drilling a hole in the cortical plate for the injection of blood.

97. Histologic Changes in Gonads of Mice Following Parotin Injections.—*Austin H. Lawrence, Yale University School of Medicine, New Haven, Conn.* Young adult dba Bar Harbor mice, weighing between 18 and 20 grams, received daily intramuscular injections of 0.125 mg. of Parotin, supplied by the Teikoku Hormone Mfg. Co. of Tokyo, Japan. Mice were killed after daily injections and testes were removed at once and fixed in Zenker's solution. Sections were cut at 5 micron and staining was with H. and E. The following phenomena were noted grossly: (1) the male mice became very vicious, and (2) the testes became enlarged. Microscopically, there was (1) hyperemia of the interstitial vessels, (2) increase in the size of interstitial spaces, which was more pronounced near main vessels, (3) increase in primary spermatocytes, often filling the lumen with these cells, and (4) increase in numbers of mitoses among the primary spermatocytes.

98. BW1081 Mouse Salivary Gland Tumor Growth Characteristics-Host Responses.—*James O. Beck, Jr., Indiana University, School of Dentistry, Indianapolis.* The BW1081 mouse salivary gland tumor, a myoepithelioma which developed spontaneously in the Roscoe B. Jackson Memorial Laboratory stock of BALB/c mouse, is tranplantable in the BALB/c mouse and kills the host in 22 to 103 days depending on the size and treatment of the inoculum, site of transplantation, and experimental conditions of the host. Metastasis has not been observed; however, a progressive central necrosis is a characteristic feature of this neoplasm. A 64-day-old tumor, weighing 11 grams, has been observed (average mouse weight, 20 to 25 grams). The myelogenous leukemoid response of the host to this neoplasm has been followed by periodic white blood cell counts. These counts range up to 450,000 cells per $mm.^3$ The spleen and liver of animals supporting high white cell counts have been markedly involved by leukemoid infiltrations and extramedullary myelopoiesis. A drastic reduction in the size of the granular tubules in the submaxillary salivary glands in adult male animals, exhibiting white cell counts of over 100,000 per $mm.,^3$ has been a rather constant observation. The tumor growth and the leukemoid response of the host have been further studied in splenectomized, adrenalectomized, gonadectomized, and desalivated animals and in animals having a combination of these operations. Splenectomy alone produces an increased tumor growth rate and an increased leukemoid response rate.

99. A Study of Anachoresis in Mice Utilizing Serratia Marcescens.—*R. B. MacIntosh, Marquette University, School of Dentistry, Milwaukee, Wis.* The clinical picture of so-called idiopathic pulpal destruction has been investigated several times in the past. The evidence thus far indicates that the destruction is bacterial in origin, the predisposing factors being pulpal irritation and a systemic bacteremia. The work undertaken here was intended to demonstrate the anachoretic effect in the skin of the mouse and, to repeat, with the use of pigment producing bacteria, the work done in this field. Under ether anesthesia, a drop of sterile croton-mineral oil mixture was injected subcutaneously into previously shaved abdominal mouse skin. Immediately, 0.1 ml. of 18- to 24-hour culture of *Serratia marcescens* was injected into the tail vein. A control series of experiments was performed under the same conditions except that sterile mineral oil and heat-killed bacteria were used. Tail blood cultures were taken at different time intervals. Some of the animals were killed and examined during the experimental period of 15 days. At post mortem, samples were obtained from the site of injection, peritoneal fluid, and heart blood for bacterial isolation and the recovery of the test microorganism. The results showed 76 per cent of cultures from the irritated site positive for *S. marcescens* as opposed to none for the control sites. The irritated site remains consistently attractive for the test microorganism for the first 8 days.

100. Carbohydrate Components of Dental Tissues.—*Ralph C. Burgess, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada.* Enamel powder, purified by the Manly-Hodge procedure, was demineralized against ethylene diamine tetracetate (pH 7.1) and separated into soluble and insoluble organic fractions by centrifugation. These fractions were hydrolyzed by a cation exchange resin (Dowex 50—X12) at 80° C. for 96 hours. Under these conditions simple sugars and uronic acids remain in solution while hexosamines can be eluted from the resin with 0.5 N HCl. The enamel hydrolysates were analyzed by paper chromatography using a pyridine; N-butanol; water solvent system (1:3:1). This method provided evidence that both soluble and insoluble organic fractions of enamel contain the sugars: galactose, glucose, mannose, and fucose. Repeated chromatographic analysis of enamel samples (up to 5 grams) gave no positive evidence that hexosamine or uronic acid was present. Similarly, the Dische reaction for uronic acid and the Elson-Morgan reaction for hexosamines gave essentially negative results when applied to enamel hydrolysates.

101. In Vitro Binding of Cations by Tissue Polysaccharides.—*D. L. Anderson, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Canada.* A conductimetric investigation of the in vitro binding of cations by hyaluronic acid, chondroitin sulphate, and heparin indicated that these polysaccharides bound cations. The number of active binding sites per unit of the molecule was calculated to be 1 for hyaluronic acid (0.0125 per cent), 1 for chondroitin sulphate (0.015 per cent), and 7 for heparin (0.01 per cent. Heparin bound the cations to a greater degree than did the other 2 polysaccharides. All 3 polysaccharides formed chelates with ferric ions, caused polymerization of methylene blue and thionine molecules, bound histamine, alcian blue and azure A. Azure A molecules polymerized on binding to heparin and hyaluronic acid but did not do so with chondroitin sulphate. Azure A, thionine and methylene blue were bound approximately twice as much by heparin and chondroitin sulphate as by hyaluronic acid. Calcium ions were bound by heparin and chondroitin sulphate but not by hyaluronic acid.

102. Influence of Caries Activity on Respiration of Salivary Microbial Flora.—*George H. Green, Dental Research Facility, U. S. Naval Training Center, Great Lakes, Ill.* The endogenous respiration and the oxidation of glucose, lactate, acetate, and pyruvate by the salivary microbial flora of 25 caries-active and 25 caries-inactive individuals were measured in a Warburg respirometer using conventional manometric technics. The relationship of these metabolic respiration rates to dental caries activity was investigated. A statistical analysis of the data failed to reveal any correlation between the endogenous respiration rates and dental caries activity. However, a statistically significant difference was found between the 2 groups in their respiration rates of glucose and lactate. The caries-active group exhibited an extremely rapid oxidation of these substrates. The caries-inactive group not only showed a much slower rate but, also, a 4- to 30-minute lag period before oxidation commenced. Acetate was not oxidized by either group. The oxidation of pyruvate was not statistically significant for either group during the first 60 minutes of incubation. After 140 minutes, however, the caries-active group showed considerable activity for this substrate. It is believed that the presence of a lag period and the rates of oxidation of glucose and lactate by the salivary microbial flora may be a true reflection of the state of dental caries activity.

103. Application of Ultrasonic Energy to Cleaning and Sterilization of Dental Instruments.—*W. G. Goodale and John J. DeGooyer, State University of Iowa College of Dentistry, Iowa City, and T. J. Bulat, Ultrasonic Laboratories, Pioneer-Central Division, Bendix Aviation Corporation, Davenport, Iowa.* A program of clinical testing and laboratory experimentation directed at an investigation of the functioning of ultrasonic energy when applied to cleaning and cold sterilization procedures has been conducted. The cleaning tests demonstrated that instruments freshly contaminated with dried blood, bone, enamel, and dentin were microscopically clean after brief immersion (an average of approximately 1 minute) in a water detergent solution activated by ultrasonic waves with a frequency rate of approximately 18,000 cycles per second. Cold sterilization experiments established the effectiveness of ultrasonic activation of bacteriocidal solutions in rapidly sterilizing instruments contaminated with microorganisms commonly encountered in clinical procedure.

104. Penetration of Cavity Varnishes by Phosphoric Acid.—*Charles E. Newcomer, State University of Iowa, Iowa City.* The penetration of 5 cavity varnishes by phosphoric acid was investigated by utilizing the radioactive P_{32} isotope to tag the phosphoric acid of dental cement liquids. Two of the varnishes were compounded from formulas contained in *Accepted Dental Remedies* (1956) and 3 were proprietary. The problem of obtaining a workable, removable film was solved by applying the varnish to nylon hose mesh. Three different test applications were made upon each of the tested varnishes: (1) the tagged liquid phase of silicate cement; (2) silicate cement mixed with tagged liquid; and (3) zinc phosphate cement mixed with tagged liquid. After the cements had thoroughly hardened, the absorbent paper under the films was analyzed with a sensitive scintillation counter to test for any penetration of the phosphoric acid. Only 2 varnishes showed any penetration. These were the result of the direct liquid application. Both of these varnishes were of a soft, gummy nature when set. Since the test did not closely approximate clinical conditions of use, it was difficult to draw any definite clinical conclusions. It does, however, give an indication of the resistance to phosphoric acid penetration of the gum resin varnishes and demonstrates that they may have value in this regard.

105. A Study of Vertical Dimension.—*Ralph C. Appleby, College of Dentistry, State University of Iowa, Iowa City.* An effort was made to permit better understanding and to analyze several of the more common methods of determining the physiologic rest position, amount of free way space, and physical contact position of young male adults. A series of tests and measurements were made on 140 young male adults, with a normal complement of teeth and good muscle control of the masticatory apparatus. The patient was seated in a dental chair, with his head upright, yet relaxed, and his body relaxed and unstrained. Two tiny dots were made on the patient's face by means of a fountain pen. One was placed just below the base of the nose (subnasion) and one on the point of the chin (gnathion). The patient was given specific instructions prior to each test for the sake of clarity and uniformity. A measurement of vertical distance betwen the 2 marks was made for each test, and taken by means of a millimeter ruler. The following tests were used: (A) "M" (phonetic test), (B) fatigue, (C) exhalation, (D) reading aloud (general reading and selected reading), (E) silent reading, and (F) swallowing movements. The "M" test proved positive on 46.6 per cent, the range of free way space was 0 to 6 mm., average free way space was 2.54 mm., and the halfway point in swallowing proved positive for the physical contact position in 50.7 per cent.

X. Cementum and Bone Grafts. March 21, Afternoon, Section A.

106. Determination of Cementum Thickness.—*Beat Hürzeler and Helmut A. Zander, Eastman Dental Dispensary, Rochester, N. Y.* A method was developed to measure cementum thickness, based upon serial horizontal sections of roots of teeth from the apex to the cementoenamel junction. The stained sections were magnified to 25× and tracings were made of the cementum and dentin. From these tracings, the cementum volume and cementum thickness were obtained by the use of mathematical computations. Cementum thickness was determined for 110 human teeth which were collected at random. They were single-rooted teeth from patients under 20 or over 50. The cementum thickness of the older age group was about 3 times that of the younger age group. From teeth with a mean age of 17, it was 0.076 mm., while that from the group with a mean age of 59 was 0.206 mm.

107. Continuous Cementum Apposition.—*Helmut A. Zander, Eastman Dental Dispensary, Rochester, N. Y.* The concept of continuous cementum formation has appeared in the literature since it was stated by Magitot. However, the rate of cementum formation is unknown. Two hundred and thirty-five single-rooted teeth, from patients ranging in age from 11 to 76, were used for this study. Cementum thickness was determined by the method described by Hürzeler and Zander. In addition to determination of average thickness for the whole tooth, determinations were also made of the cementum thickness at the apical 8/10 mm., the mid-portion of the root, and the area near the cementoenamel junction. The statistical analysis of these data shows a significant continuous increase in thickness of cementum with age in all the areas studied. There is a straight-line relationship between the cementum increase and age. The steepness of the line varies for different groups of teeth and for the different areas of a tooth, but they are all interrelated.

108. Response of Oral Tissues to Grafts of Heterologous Anorganic Bone. II. Host Response to Implants of Anorganic Bovine Spongy Bone.—*Harvey W. Lyon and Fred L. Losee, Dental Division, Naval Medical Research Institute, Bethesda, and Philip J. Boyne, Marine Corps Base, 29 Palms, Calif.*

Previous reports have shown that there is biologic "take" of anorganic bovine cortical bone by satisfying 2 major criteria for a successful transplant, namely: incorporation of implant without subsequent foreign body reaction, and formation of typical callus followed by bony union with the host. A continuation of this study was undertaken to determine whether implants of heterologous anorganic spongy bone obtained from ethylenediamine-treated bovine femoral heads fulfill the aforementioned requirements. Implants of anorganic spongy bone were inserted into typical exodontic defects in adult Rhesus monkeys and dogs in an attempt to restore contour to alveolar ridges from bone loss due to multiple extractions, fractured buccal plates, and simulated cystic defects. Postoperative 13-day partial resections demonstrated host biologic acceptance with subsequent bony union. Ridge contour was restored and primary observations indicate definite elevation in height of the bony crest.

109. Response of Oral Tissues to Grafts of Heterologous Anorganic Bone. III. Human Host Response to Implants of Anorganic Bovine Bone.—*Philip J. Boyne, Marine Corps Base, 29 Palms, Calif., and Harvey W. Lyon and Fred L. Losee, Dental Division, Naval Medical Research Institute, Bethesda.* A clinical evaluation of heterogenous bone implants in man was undertaken to parallel animal experimentation. Implants of anorganic bovine cortical bone and anorganic spongy bone taken from bovine femoral heads were placed in oral bony defects of 50 human patients in an effort to create an environment for rapid osseous healing and to maintain alveolar ridge height and contour. The defects used in this study had resulted from the destruction of alveolar bone by the commonly occurring periapical and periodontal pathologic lesions, and from loss of osseous structure attending complicated exodontia. The postoperative course was uniformly uneventful. There was no evidence of foreign body reaction. The clinical and roentgenographic evidence indicated a pattern of host acceptance similar to that observed in corresponding defects in the Rhesus monkey. Clinical observations during the 6- to 12-month postoperative period indicate that it is possible to maintain alveolar ridge contour and height by the use of anorganic bone heterografts.

110. Histologic Comparison of Bovine and Anorganic Bone Implants in Human Beings.—*William H. Bell, Edward C. Hinds and Sumter S. Arnim, The University of Texas Dental Branch, Houston.* Bovine and anorganic bone chips were placed into extraction sockets of human clinical patients. Some of the sockets were allowed to fill with blood and these constituted controls. Periodic biopsies were taken from the healing extraction sockets. Biopsies, taken after 2 weeks, from tooth sockets containing bovine bone chips revealed many polymorphonuclear leukocytes. Multinucleated giant cells and many round cells were found after 5 weeks. Biopsies from tooth sockets containing anorganic bone chips showed considerably less inflammatory reaction. Many of the macrocanalicular systems were vascularized after 7 days. As healing progressed, reactive new bone formation took place around remnants of many of the anorganic chips. These results indicate that anorganic bone chips are better accepted by the host than the cultured bovine bone chips.

111. Study of Polyvinyl Resin Sponge in Jaws of Dogs.—*William J. Dresser and Henry B. Clark, Jr., School of Dentistry, University of Minnesota, Minneapolis.* A study was performed to determine the degree of acceptance and osteogenetic potential of polyvinyl resin sponge (Ivalon) in the jaws of dogs. Seven animals were used. Pieces of this material were implanted in previously prepared maxillary and mandibular edentulous alveolar ridge sites. Seven implants were laid over cortical bone (onlays) and covered with mucoperiosteum,

and 9 were placed in prepared defects in medullary bone (inlays). No controls were used. The implants were observed grossly in the living animals for periods of 4 to 28 weeks prior to sacrifice. Five of 7 onlays and 4 of 9 inlays remained healthy until removed. The remainder were lost due to separation of the wound margins. Histologic study of the grossly successful implants showed minimal foreign body reaction and satisfactory acceptance by the host tissues. The spaces within the sponge became filled with fibrous connective tissue, and in the two 28-week specimens, were partially infiltrated with bone.

112. The Use of Inorganic Beef Bone Matrix in Oral Surgery.—*James R. Hayward, Emmett R. Costich, and James K. Avery, University of Michigan, Ann Arbor.* Ethylenediamine extracted beef bone has been utilized to eliminate bony defects following various surgical procedures, as an hemostatic agent, and as a grafting material for the correction of micrognathia, and for the elimination of undesirable undercuts in alveolar ridges. Sections of medullary bone have been found most desirable, although they are more difficult to handle due to the extreme friability of the inorganic matrix. The patients have been followed by periodic x-ray examination. This has revealed regeneration of new bone in the spaces not occluded by the inorganic bone matrix. Postoperative films before placement of the bone and immediately after the bone is placed are necessary for evaluation of succeeding x-rays. There have been no postoperative complications following the use of this material that could be attributed to the bone matrix.

113. Autogenous Bone Grafts in the Treatment of Intrabony Periodontal Pockets.—*Isra Yuktanandana, University of Alabama, School of Dentistry, Birmingham.* The present study was undertaken to determine whether reattachment or adaptation of tissue to the root surface takes place following the insertion of autogenous bone graft into intrabony periodontal pockets. Intrabony pockets were surgically produced on both sides of the maxillary arch of 10 dogs and were packed with various foreign bodies until pathologic changes occurred. Autogenous, cancellous bone chips were then inserted on the experimental side while the other side was left as control. The animals were sacrificed at varying periods from 1 week up to 16 weeks. Clinical and roentgenographic observations were correlated with histologic findings. The results indicate that, under favorable circumstances, the intrabony pocket can be eliminated by bone graft procedure. Bone regeneration and connective tissue reattachment to the tooth surface occur in the presence of the graft and healing takes place by attachment of new periodontal fibers upon new cementum. The control side without the bone graft heals by adaptation of an extensive epithelial attachment to the root surface, and only a relatively small amount of bone regeneration occurs.

114. Healing of Artificial Pockets in Dogs.—*Nicholas R. Marfino, Frank M. Wentz, and Balint J. Orban, Loyola University, Chicago College of Dental Surgery, Chicago, Ill.* The aim of this investigation is to study the regenerative capacity of the supporting tissues of the teeth of dogs. Surgical flaps were made on the upper canines of 9 young mongrel dogs. Notches were cut into the teeth at the margin of the gingiva and at the bottom of the surgically produced pocket. The creation of the pocket entailed removal of a 7 by 6 mm. section of alveolar bone. The wound was packed with zinc oxide and eugenol. The pack was retained for 3 weeks until the pocket had epithelized and was then removed. The animals were sacrificed from 1 to 11 months after surgery. Good morphologic and functional re-establishment of a new dentogingival junction was observed. A new formation and attachment of connective tissue

elements to the tooth surface was seen early in the most apical portion of the experimental pocket. The epithelium, lining the inner surface of the pocket, attached itself to the surface in most experiments. This attachment was coronal to the area of attachment of new connective tissue and was observed in later stages of the healing process.

115. Preliminary Observations Concerning Supra-alveolar Fibers After Orthodontic Treatment.—*H. E. Thompson, Hugh I. Myers, J. M. Waterman, and Viola D. Flanagan, University of Kansas City, School of Dentistry, Kansas City, Mo.* Three groups of monkeys were studied: (1) two normal animals with no orthodontic tooth movement, (2) two animals in which a tooth was removed and the teeth adjoining the space were moved into the space but not quite approximated, and the animal sacrificed, and (3) one animal in which a tooth was removed and the teeth adjoining the spaces were approximated. Following approximation, the supra-alveolar fibers were completely gingivectomized. After healing for 3 weeks, all orthodontic appliances were removed and the animal sacrificed 17 weeks later. All the jaws were formalin-fixed, decalcified, paraffin embedded, sectioned at 10 micra, and every tenth section mounted and stained with hematoxylin and eosin. The connective tissue above the alveolar crest (alveolar fibers) showed complete regeneration in both Groups 2 and 3 animals. The density of the fibers seemed to be the same in all 3 groups.

116. Heterotopic Osteogenic Effect of Calcium Hydroxide in Rat Connective Tissue.—*David F. Mitchell, Indiana University, School of Dentistry, Indianapolis.* In surveying the rat connective tissue reaction to implanted pellets of various dental materials it was noted that calcium hydroxide (pH 12.3) and water pellets induced heterotopic osteoid tissue in the capsule around the pellet. This reaction subsequently was found around duplicate specimens in 13 of 17 rats studied at 17 different intervals from 2 to 200 days. Pellets of methyl cellulose and calcium hydroxide gave similar reactions; 6 intervals, 3 to 33 days. Calcium hydroxide and Gelfoam revealed osteoid after 26 days, but not after 17. Barium hydroxide (pH 12.6) caused acute severe abscess formation and no osteoid. Other materials tested over at least 3 intervals, up to 39 days, included aluminum hydroxide (pH 7.1), magnesium oxide (10.3), monobasic calcium phosphate (3.5), barium sulfate (6.1), and tribasic calcium phosphate. In only one instance ($BaSO_4$, 20 days—1 out of 6 tested intervals) was osteoid found. Despeciated calf bone paste was studied after implanting fresh and dried material for 7 intervals (3 specimens each) from 15 to 71 days. Anorganic bone was also studied. These 2 materials, as studied, did not appear to induce heterotopic bone as consistently or in as great amount as did calcium hydroxide.

XI. Microbiology. March 21, Afternoon, Section B.

117. Time-Function Studies of pH and the Uptake of Potassium, Sodium, and Phosphorus During the Growth Cycle of *Lactobacillus acidophilus* (ATCC 4356).—*Norman O. Harris, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.* Automatic pH recordings have been made of growing cultures of *L. acidophilus* and correlated with time-function studies of potassium uptake. Characteristic pH curves can be associated with the age of the inoculum, being more convex with younger organisms. The envelope of potassium uptake curve is characteristic, coinciding in increase with the logarithmic phase and thence returning to zero. Peak potassium uptake of 24- to 48-hour culture occurs at approximately 12 to 16 hours and may range

from 0.2 to 1.6 mEq./1. Levels of uptake are reproducible when the same inoculum is used, but the magnitude at peak uptake can vary considerably when different inoculums of the same species and origin are utilized. The envelope of the sodium and phosphorus uptake curves do not coincide with the potassium curve, sodium peaking and remaining high while phosphorus peaks and then drops to a plateau.

118. The Effect of Sodium Fluoride on Extracts of Fluoride Resistant Lactobacillus Casei.—*Robert E. Gillis, Western Reserve University, Cleveland, and Seton Hall College of Medicine and Dentistry, Jersey City.* Prior experimental results indicated that fluoride-grown cells metabolize glucose in the presence of fluoride via the same pathway as normally grown cells in the absence of fluoride. We have advanced several hypotheses to explain this phenomenon: (1) the existence of an alternate, fluoride-resistant pathway of glycolysis, (2) the existence of a fluoride-resistant enolase, and (3) an alteration in the permeability of fluoride-grown cells to fluoride. Experiments designed to test the efficacy of these hypotheses have revealed that glycolysis of cell free extracts of fluoride-grown cells is markedly inhibited by added sodium fluoride, and that the enolase reaction in partially clarified extracts is completely inhibited by this agent. This suggests that the first 2 hypotheses advanced above are unlikely. Preliminary results of studies initiated to examine the third hypothesis suggest that the fluoride-resistant glycolysis of fluoride-grown *Lactobacillus casei* is the result of some alteration in the permeability of this organism to the inhibitor.

119. Microbial Decomposition of Enamel and Dentin in vitro.—*Arthur N. Bahn, College of Medicine, Irwin Weinstein and Maury Massler, College of Dentistry, University of Illinois, Chicago.* The acidogenic and proteolytic activities of *Streptococcus liquefaciens, Strep. viridans, Lactobacillus acidophilus,* and *Clostridium perfringens* were studied by placing molar crown quadrants in various media inoculated with each organism. Fifty-six freshly extracted teeth were cleaned, quartered, weighed, and sterilized, then placed in media rich in carbohydrate only (sucrose), protein only (casein hydrolysate), a mixture of both, and in Brewer's thioglycollate, inoculated with one of the test organisms, and incubated. Media were renewed every 72 hours. Weight loss in each of the specimens and hydrogen ion concentration changes in the media were analyzed. Six replicates and 1 control were used in each series. With all the test organisms the greatest mean weight loss occurred after 8 weeks in the media rich in both carbohydrate and protein. Little or no weight loss occurred in the medium containing protein only. *L. acidophilus* produced the greatest mean weight loss in this series. Acid production was significant in all media containing carbohydrate, but was minimal in the medium with protein only. Weight loss was not directly correlated with acid production. These data suggest that proteolytic activity may play a role in the microbial decomposition of enamel and dentin.

120. Spreading Factor Activity of Bacterial Chondrosulfatase.—*Stephan E. Mergenhagen and Henry W. Scherp, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Chondrosulfatase produced by a microaerophilic diphtheroid bacillus, isolated from the gingival sulcus, appears to increase tissue permeability as does hyaluronidase. Three strains of this bacillus produced 25 turbidity reducing units (TRU) of chondrosulfatase per milliliter in a Casitone-yeast extract broth after 72 hours' anaerobic incubation. When injected into rabbit skin, with India ink as indicator, supernates of such cultures produced areas of diffusion from 2 to 3 times as great as those produced by sterile broth or by supernates inactivated at 100° C. for 10 minutes.

Furthermore, incorporation of glucose in the broth, although it greatly increased growth of the diphtheroid bacillus, abolished formation of both chondrosulfatase and spreading factor. In comparison, a culture filtrate of *Staphylococcus aureus,* containing 40 TRU of hyaluronidase per milliliter, effected only half as much spreading. Accumulation of intravenously administered Evans blue in areas of intracutaneously injected chondrosulfatase preparations provided evidence of increased permeability of vascular connective tissue. These actions of bacterial chondrosulfatase may be significant in the pathogenesis of periodontal disease.

121. Depolymerization of Ground Substance in Experimental Infections.—*John C. Thonard and Henry W. Scherp, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* To ascertain whether spread of certain infections is accompanied by depolymerization of ground substance, wattles of roosters were injected interdermally with 1 ml. of cultures of a non-hyaluronidase-producing oral anaerobic streptococcus (S.M.), a hyaluronidase-producing oral anaerobic streptococcus (JR4), and a hyaluronidase-producing hemolytic staphylococcus (SAB2), individually, in combination, or with testicular hyaluronidase, viridans streptococcus hyaluronidase, or SAB2 filtrate. The paired wattle injected with heat-inactivated bacteria or hyaluronidase preparations served as control. After 48 hours, tissues were fixed in formalin, sectioned, and stained by H. and E., Hale's, periodic acid-Schiff, and toluidine blue technics. Control sections were incubated with active and inactivated testicular hyaluronidase before staining. Infections by in vitro hyaluronidase-producing organisms, singly or combined, showed complete absence or marked decrease in the Hale-positive and metachromatic material of the dermis. Injections of strain S.M. (0.5 ml.) with hyaluronidase preparations (0.5 ml.) gave equivocal results probably due to insufficient dosage. Sections demonstrating Hale-positive material lost this staining when incubated in saline solutions of either active or inactivated testicular hyaluronidase. Contrariwise, although aqueous solutions of testicular hyaluronidase removed the Hale-positive staining, heated aqueous hyaluronidase did not. The experiments show that (1) rooster wattles will support experimental infections, (2) hyaluronic acid depolymerization in vivo may be a factor in the pathogenesis of these infections, and (3) the present use of enzymes, in histochemistry, for identifying specific tissue components in tissue sections needs careful re-evaluation.

122. Effect of Hyaluronidase on Hemolytic Staphylococcus Abscesses in Hamsters.—*J. M. Waterman, Hugh I. Myers, and Viola D. Flanagan, University of Kansas City, School of Dentistry, Kansas City, Mo.* Preliminary treatment of the animals consisted of placing the tissue on the back of each hind leg immediately above the knee between the jaws of pliers and producing trauma with a weight attached to the handle. Three groups of animals were used: (1) right leg injected with bacterial suspension and normal saline and left leg injected with bacterial suspension plus hyaluronidase, (2) right leg injected with bacterial suspension and normal saline and left leg injected with saline, and (3) right leg injected with bacterial suspension, 15-minute delay, then normal saline injected and left leg injected with bacterial suspension 15-minute delay, then hyaluronidase. The animals were sacrificed after 48 hours. Histologic sections were prepared from the specimens taken to include tissue well beyond the traumatized area. Cultures were made of each suspension used on preparation and on completion of its use. Results indicate that hyaluronidase does not effect viability of the hemolytic coagulation plus staphylococcus on blood agar plates, nor in abscess formation in the animal. This differs from the results previously found using a hemolytic streptococcus.

123. The In Vitro Inhibition of Beta Hemolytic Streptococci by Saliva.—*Henry A. Bartels, Harry Blechman, and John Cavallaro, Murry and Leonie Guggenheim Foundation, Institute for Dental Research, College of Dentistry, New York University, New York.* Since beta hemolytic streptococci appear only as occasional members of the oral microbiota, saliva was examined to determine if a specific anti-hemo-streptococcal factor was present. Heated and unheated saliva, 25 per cent by volume, was added to a basal medium of brain heart infusion agar. The surface was streaked with test microorganisms: *Micrococcus lysodeikticus, Micrococcus aureus, Bacillus cereus, Neisseria flavescens* and various strains of beta hemolytic streptococci. Over 200 specimens of saliva collected from 16 different normal individuals at various times were examined. It was found that an anti-beta hemolytic streptococcal factor was present in fresh saliva. The factor is distinct from lysozyme, not filterable through the Seitz sterilization filter, and is associated with the oral microbial flora. It inhibits the growth of both stock and recent beta hemolytic streptococcal isolates. Since this action is manifested in the presence of laked blood, potato juice, lyophilized catalase, and under anaerobic conditions, we conclude it is distinct from the hydrogen peroxide production by oral microorganisms.

124. Frequency Distribution of Lactobacilli From the Hamster.—*Morrison Rogosa, Murray Disraely, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda, and Erling Johansen, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* Oral samples were obtained using quantitative technics. Seventy-three animals were sampled at weaning and also 7 days later. Three hundred and thirty isolates from representative colonies on SL agar were studied. Seven different homofermentative and 4 heterofermentative species of Lactobacillus (L.) were characterized. Of these, 7 appear to be hitherto undescribed or new species, of which 5 are homofermentative and 2 are heterofermentative. *L. salivarius* comprised 32.1, *L. acidophilus* 7.3, *L. brevis* 26.7, and *L. fermenti* 7.3 per cent of the isolates, respectively. The apparently new species were 26.6 per cent of the total. The distribution of species was widely different from that in man. *L. casei, L. plantarum, L. arabinosus, L. cellobiosus,* and *L. buchneri,* comprising 48.6 per cent of the lactobacilli in man, were not present in the hamster. Furthermore, none of the 7 new hamster species was present in man. Also, oral and fecal lactobacilli were not significantly different from each other.

125. Further Studies on Lactobacilli Counts After Elimination of Carious Lesions.—*Robert G. Kesel, The University of Illinois, College of Dentistry, Chicago, Irving L. Shklair, George H. Green, and Harold R. Englander, Naval Dental Research Facility, Great Lakes, Ill.* Five males, average age 25 years, who had at least 10 carious lesions and high salivary lactobacilli counts were selected. Four pre-counts were made on tomato and Rogosa's media from each person. The average pre-count for the group was 74,000 col./ml. saliva. All carious lesions were filled with amalgam or silicate. Lactobacilli counts were made thereafter at weekly intervals for periods of from 10 to 18 months. The post-counts of all patients dropped to levels below 900 col./ml. during the first postoperative week and remained at this level. Two of the 5 patients exhibited zero counts throughout the postoperative period of observation. The carious lesions of 10 naval recruits, average age 18 years, with rampant caries and high lactobacilli counts were filled with zinc oxide and eugenol cement. Post-counts were made at weekly intervals for 4 weeks. Seven of the 10 subjects showed a zero count during the first postoperative week and 4 remained at this level throughout the 4-week period. Newly formed cavities and decalcifications were observed in those cases which did not

show reductions. Lactobacilli counts of persons with rampant caries were made before, after full mouth extraction before the insertion of artificial dentures, and after the insertion of artificial dentures. After all teeth were extracted, the counts were reduced to zero and remained at this level until about 6 weeks after the insertion of artificial dentures.

126. MANOMETRIC STUDIES OF MICROORGANISMS IN HUMAN PAROTID SALIVAS.—*Sterling W. Jackson, George I. Baxter, William J. Kimber, and Ned B. Williams, University of Pennsylvania, School of Dentistry, Philadelphia.* Culture work demonstrated that oral strains of *Monilia albicans* (A), *Aerobacter cloacae* (B), and *Streptococcus faecalis* (C) survived in human sterile parotid saliva, but an alpha streptococcus (D) and a homofermentative lactobacillus (E) did not. Manometric technics using a Warburg apparatus were applied to the study of O_2 uptake of washed, resting cells in samples of human sterile parotid saliva. All saliva was collected aseptically using parotid cups. The following mean values, in microliters of O_2 uptake per milligram of cell N, were taken from the respiration curves of each strain after 90 minutes at 35.0° C.:

	A	B	C	D	E
Saliva	64	28	3.0	4.0	2.1
Endogenous	52	10	0.84	1.6	0.75

At any given time the endogenous values were always less than the O_2 uptake of microbes in saliva. Microbes A and B show greater oxgen consumption than either C, D, or E, even though the values are different for each microbe. However, all levels are far below those expected for microbes metabolizing in an optimal environment. It is concluded that human sterile parotid saliva is not an optimal environment, but contains components which can be utilized in metabolism.

127. AN ELECTRON MICROSCOPE STUDY OF PARTICULATES IN PERIODONTAL EXUDATE.—*L. McMillan, D. Y. Burrill, and L. S. Fosdick, Northwestern University Dental School, Chicago, Ill.* Individuals with periodontal disease and who had deep suppurative periodontal pockets were selected. After surface debris was washed away with a stream of water, exudate from suppurative pockets was collected in a polyethylene capillary tube, or it was collected from the pocket by gently scraping the soft tissue wall of the pocket with a blunt and smooth periodontal membrane explorer. Each sample was placed in distilled water, homogenized by shaking, and a portion of the mixture examined with a light microscope. If large clots or cell debris were present the sample was centrifuged and washed and again diluted and viewed with a light microscope to determine microbial density. The density was adjusted to that proper for the electron microscope and portions of the sample were transferred to previously prepared Form Var treated screens. After light chromium shadowing the particles were viewed and photographed with the electron microscope. Organisms present constantly included spirochetes, cocci, and bacillus forms. Pus cells and leukocytes were too large to be observed. In one sample of exudate collected by the tube, and in most samples collected by scraping the wall of the pocket, fibers of collagen were found.

128. EFFECTS OF GLUCOSE, LACTATE, AND pH ON CERTAIN BACTERIAL AND CHEMICAL CHANGES IN INCUBATED SALIVA.—*T. Shiota, American Dental Association at the National Institute of Dental Research, and M. F. Kunkel, Jr., National Institute of Dental Research, National Institutes of Health, U. S. Public Health*

Service, Bethesda. The effects of glucose, sodium lactate, and pH on certain bacterial and chemical changes in incubated pooled saliva were studied. The saliva pool was divided into 6 lots. Glucose was added to lots 1 and 2 to make 0.2 and 0.5 per cent final concentrations, respectively. Lots 3 and 4 received sodium lactate to make final concentrations of 0.2 and 0.5 per cent, respectively. The pH of lot 5 was adjusted to 6.5, whereas lot 6 was not altered (pH 7.7). Upon incubation, the unaltered saliva supported the growth of fusobacteria, but not lactobacilli, streptococci, or Veillonella. The addition of glucose increased the lactobacillus populations, whereas the pH 6.5, pH 7.70, and the lactate added lots showed no such increase. Glucose brought about a rapid decline in the fusiforms in contrast to the other treatments. The streptococci increased with the addition of glucose, whereas the addition of lactate and the lowered pH maintained the streptococci for a longer period than the unaltered (pH 7.7) saliva. The Veillonella increased rapidly in the presence of glucose or sodium lactate. The ammonia and indole formation was inhibited completely by both concentrations of glucose and, to a slight extent, by 0.5 per cent sodium lactate. The total nitrogen of the pooled saliva was 700 μg/ml., free ammonia nitrogen 125 μg/ml., carbohydrate as glucose, 215 μg/ml., and the dry weight, 7 mg./ml.

XII. Experimental Caries and Salivary Gland. March 21, Afternoon, Section C.

129. Relation of Feeding Regimens to Dental Caries in Rats.—*Rachel H. Larson, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* In order to study the effect of (1) limited vs. unlimited feeding and of (2) injected Na_2EDTA, 36 litters of 5 rats each were placed on Diet 633, 2 rats per cage, under the following regimens: (A) 8 hour ad libitum, Na_2EDTA injected, (B) 24 hour ad libitum, Na_2EDTA injected, (C) 8 hour ad libitum controls, (D) controls "pair fed" to Group B, diet given in 3 aliquots per week, and (E) 24 hour ad libitum controls. The results include the following: percentage incidence of caries, (A) 3.4, (B) 80.8, (C) 0.0, (D) 38.2, and (E) 65.7; av. carious teeth, (A) 0.03, (B) 3.0, (C) 0.0, (D) 1.0, and (E) 2.2; av. carious lesions, (A) 0.03, (B) 4.2, (C) 0.0, (D) 1.0, and (E) 2.3; av. caries score, (A) 0.03, (B) 7.1, (C) 0.0, (D) 1.7, and (E) 4.2; av. grams daily food consumption per rat, (A) 7.7, (B) 10.4, (C) 10.4, (D) 10.2, and (E) 14.7. A study of the incidence and extent of caries shows significant differences between (1) the 8- and 24-hour injected groups, (2) the 8-hour controls and the "pair-fed" controls (food intake equal), and (3) the "pair fed" and the 24-hour controls. There was no significant difference between either of the Na_2EDTA injected groups and their controls fed for an equal length of time. These results emphasize the importance of the length of time that food is in the oral cavity and that this effect is not necessarily related to total intake.

130. Studies on the Heat Treatment of a Cariogenic Diet.—*Harold V. Jordan, Robert J. Fitzgerald, and William L. Poole, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* It has been reported that autoclaving of diets containing skim milk powder results in an increase in smooth surface caries and a decrease in weight gains in rats. Supplementation of the diet with lysine tended to counteract these effects. In the present studies, autoclaving of a coarse particle diet (585), which produces occlusal fissure caries in Sprague-Dawley rats, resulted in reduced growth for experimental periods up to 90 days but there was no change in the amount of caries produced. The composition of this diet is 30 per cent dried whole milk

powder, 42 per cent yellow hominy corn grits, 25 per cent granulated cane sugar, 2 per cent whole dried liver substance, and 1 per cent salt mixture. Addition of the members of the B vitamin complex to the drinking water, individually or in combination, had no effect on the growth of the rats, while the combination of thiamine, riboflavin, and folic acid resulted in increased caries scores. Supplementation of the autoclaved diet with 1 per cent yeast extract resulted in a 30 per cent increase in weight gains. Addition of 1 per cent lysine to the autoclaved diet supplemented with yeast extract had no further effect on weight gains. When individual components of the diet were omitted prior to autoclaving and then restored and fed to rats for 30 days, it was found that the diet in which only the whole milk powder was unautoclaved resulted in a weight gain two thirds that found in animals receiving unautoclaved diet 585. A diet composed of equal parts of autoclaved and unautoclaved diet 585 resulted in a weight gain equal to one half that found in animals receiving unautoclaved diet 585.

131. Influence on Experimental Dental Caries After Extraction of Opposing Molars.—*C. E. Klapper and Everett Wilkie, University of Alabama, School of Dentistry, Birmingham.* All of the maxillary molars were removed from 10 partially desalivated hamsters. The mandibular molars were removed from a second group of animals. No teeth were extracted from 10 other animals which served as a control group. After being maintained on a cariogenic diet for 60 days, the caries scores of all animals were determined. Rampant caries had developed in all of the molars present in the oral cavities of these animals. To study the early stages of decay under these conditions, the experiment was repeated, but terminated at the end of 34 days. Significantly different caries scores resulted in all groups after this short period of time. In both groups where opposing molars had been removed, the caries scores were more than twice as large as in the control group. From these results it might be concluded that the factors resulting from interdigitation of opposing molars are important in either retarding the initiation of dental caries or reducing the rate of decay during the early stages. Eventually under these conditions the amount of tooth decay becomes equal in animals with or without opposing molars.

132. Caries in Rats Receiving High Sucrose Diet During Calcification of Teeth.—*Benjamin F. Watson and Joseph C. Muhler, Indiana University, Bloomington.* The purpose of this was to determine the results of feeding a high carbohydrate diet (67 per cent) on dental caries in the offspring of rats subsisting on the carbohydrate diet during pregnancy and lactation as compared to similar rats subsisting on a stock corn diet. Group I was composed of female rats which were bred while subsisting on the high sucrose diet and which remained on this diet for the duration of the experiment. Group II animals were raised on a stock corn diet, and the pups similarly remained on this diet throughout the study. Groups III and IV were composed of littermates separated at 21 days from Groups I and II, and one half of each litter remained on the diet of the mother while the other was placed on the opposite diet. The animals were sacrificed at 121 days. The caries experience of the offspring born on the carbohydrate diet and which remained on that diet throughout the study was significantly less than littermates switched to the carbohydrate diet at weaning.

133. Effect of Carbohydrates on Salivary Gland Function in the Rat. —*Richard P. Elzay and Joseph C. Muhler, Indiana Universiy, Indianapolis.* The ingestion of excessive amounts of refined carbohydrate has been repeatedly suggested as a contributing factor to the initiation of human dental caries. Recently, it was suggested that high amounts of carbohydrates may alter the viscosity of saliva, and so it was decided to investigate this effect in laboratory

animals where the experimental conditions could be carefully controlled. The diets varied in sucrose content from 20 to 60 per cent. Salivary flow was measured by stimulating gland activity with pilocarpine, and viscosity determined by special technics developed for this study which essentially involve measuring rate of passage of saliva through a calibrated tube. The results indicated no difference in salivary flow, but marked difference in salivary viscosity. The higher the carbohydrate content of the diet the lower the salivary viscosity.

134. Further Studies on Salivariadenectomized Hunt-Hoppert Rats.—*S. Rosen, H. R. Hunt, and C. A. Hoppert, Michigan State University, East Lansing, and L. M. Sreebny and E. Bachem, University of Illinois Dental School, Chicago.* This study, a continuation of work reported previously, was an experiment on selective salivariadenectomy of caries-resistant rats. In one group of animals, the parotid ducts were removed and the remaining ends of the ducts were ligated. The submaxillary and sublingual glands were removed from another group of animals. A third group was used as controls. To date, animals with the submaxillary and sublingual glands removed resemble the salivariadenectomized animals of the previous experiment whereas animals with the parotid ducts removed resemble control animals. That is, animals with the submaxillary and sublingual glands removed show a caries time and a lactobacillus count similar to "completely" salivariadenectomized animals. There is no difference in caries time and lactobacillus count between animals with the parotid ducts removed and controls.

135. Ligation Atrophy of the Submaxillary Gland of the Rat.—*Theodore E. Bolden, Seton Hall College of Medicine and Dentistry, Jersey City, Julia Meyer and Joseph P. Weinmann, University of Illinois College of Dentistry, Chicago.* The excretory duct of the right submaxillary gland was ligated in 30 male Sprague-Dawley rats, 125 to 150 days of age. The left nonligated gland served as control. The animals were killed in groups of 5 at 1, 2, 3, 5, 7, and 9 days, postligation. Dry weight determinations were made on control and duct-ligated submaxillary glands. A quantitative estimate was made of the relative areas in sections of the glands which were occupied by normal acinar and by intralobular duct tissue. For this purpose, normal acini were defined as acinar cells in whose cytoplasm secretory granules were still discernible. An ocular grid divided into 66 squares was used, 1,320 squares being counted on each section. The number of squares in which half or more of the area was occupied by normal acini was counted. A similar count was made for the squares occupied by intralobular ducts. Counts were made on 3 ligated specimens from each postoperative period and a number of patent control glands. Reduction of dry weight occurred in all ligated glands. It was steepest between the third and seventh day and amounted to a loss of between 64 per cent and 73 per cent of the preoperative weight by the ninth day. The normal gland consisted of about 60 per cent acinar tissue, 25 per cent intralobular duct tissue, and 15 per cent nonsecretory tissue. By the ninth day postligation, the gland was composed of 11 per cent normal acinar tissue, 40 per cent intralobular duct tissue, and 49 per cent nonsecretory tissue plus compressed acinar cells.

136. Comparison Between Salivary Gland Extirpation and Duct Ligation on Caries.—*William G. Shafer, Joseph C. Muhler, and David Bixler, Indiana University, School of Dentistry and Department of Chemistry, Indianapolis and Bloomington.* Ligation of the major salivary gland ducts has been studied as a possible substitute for salivary gland extirpation during the course of experiments dealing with the salivary gland-dental caries-endocrine system

relationship. An unusual incidence and distribution of carious lesions were found in rats whose salivary gland ducts were ligated as compared to those in which the glands were extirpated. Two series of animals were utilized and treated in an essentially identical manner; the study was duplicated only for the purposes of confirmation. The dental caries experience in Series I expressed as mean number of lesions was: Controls—6.9 ± 1.0; Desalivates—10.2 ± 1.4; Ligates—13.9 ± 0.7. In Series II: Controls—8.8 ± 1.2; Desalivates—12.3 ± 1.5; Ligates—16.7 ± 0.6. The significant increase in caries in the duct-ligated animals, as compared to the desalivated animals, was accounted for by an increase in lesions in the maxillary arch.

137. The Effects of Extirpation and Ligation of the Salivary Glands on Organ Weights in Caries-Resistant and Caries-Susceptible Rats.—*Leo M. Sreebny and Erica Bachem, University of Illinois College of Dentistry, and Sam Rosen, H. R. Hunt, and C. A. Hoppert, Michigan State University, East Lansing.* In male and female rats, 30 to 40 days of age, of the Hunt-Hoppert caries-susceptible and caries-resistant strains, the submaxillary and sublingual salivary glands were extirpated and the ducts of the parotid gland were ligated and sectioned. The animals were then placed on a cariogenic diet. Littermate animals were pair fed with the operated group. Another group of normal control rats were fed the cariogenic diet ad libitum. There were 8 to 15 animals in each group. Caries-susceptible animals were sacrificed at 165-175 days of age; animals of the caries-resistant strain were sacrificed at 350-400 days of age. Total body weight and wet weights of the following organs were recorded: thyroid, pancreas, adrenal, testes, ovary, hypohysis, kidney, and uterus. The average weight of the pair-fed controls was nearly the same as that of the ad libitum fed animals, indicating that the food intake of the experimental group was close to normal. The body weight of the experimental animals was smaller than that of the pair-fed group. The relative weights of the adrenals and testes were greater than those of their pair-fed or ad libitum controls. No significant changes in relative weight were observed in the other organs. The smaller weight of the desalivated animals compared to their pair-fed controls may be due to impaired intestinal absorption. It is possible that the increased weight of the adrenal gland indicates response to stress caused by starvation.

138. Effect of Thyroxine on the Weight of Submaxillary Salivary Glands.—*Herbert Wells and Paul L. Munson, School of Dental Medicine and Medical School, Harvard University, Boston.* The effects of various endocrine, pharmacologic, and surgical treatments on the weight of the submaxillary salivary glands of the rat are being investigated as part of a study of the mechanisms regulating salivary gland function. In the present series of experiments, male albino rats, 170-190 Gm. in weight, were injected daily with L-thyroxine, 100 μg/100 Gm., intraperitoneally. After 14 days, the absolute weights of the submaxillary glands of the injected rats were on the average 18 per cent heavier than controls ($P < .01$). The effect of thyroxine occurred in spite of a marked depression of the over-all growth rate and was even more striking when the ratios of gland weight to body weight were compared. In similar experiments, L-triiodothyronine and L-triiodothyroacetic acid also increased the weight of the submaxillary glands. After gonadectomy, male rats failed to gain in body weight as rapidly as sham-operated controls and their submaxillary glands were proportionately smaller. Treatment of the gonadectomized rats with L-thyroxine resulted in a further depression in the over-all growth rate, but the effect on the submaxillary glands was as great as in intact rats. In these experiments, the effect of thyroxine was not dependent on the presence of the testes or of testosterone.

139. Growth Hormone, Thyroxine, and the Submaxillary Salivary Gland of the Rat.—*David Bixler and Joseph C. Muhler, Indiana University School of Dentistry, Indianapolis.* Male albino rats which were hypophysectomized at 4 months of age were divided into 4 groups. Group 1 received daily subcutaneous injections of 1 mg./Kg. growth hormone. Group 2 was given 20 μg of sodium thyroxine subcutaneously each day. Group 3 received both growth hormone and thyroxine in the concentrations already described. Group 4 received no hormones or special supplements. Unoperated rats of the same age were employed as controls. All animals were fed a special diet of bread, oranges, carrots, and milk ad libitum. After 24 days, the animals were sacrificed by chloroform inhalation. One submaxillary gland from each animal had its proteolytic activity assayed. The remaining submaxillary gland along with the sublingual and parotid glands were fixed in Bouin's fluid for histologic study. Hypophysectomy markedly reduced the submaxillary gland proteolytic activity. Histologic examination of this gland revealed atrophic changes, particularly of the granular tubules, characteristic of hypophysectomized animals. Thyroxine or growth hormone alone were only partially effective in preventing the atrophic changes of hypophysectomy. Thyroxine and growth hormone in combination, however, were completely effective in preventing hypophysectomy-induced changes. These results suggest that the effects of the various hormones on the submaxillary gland are nonspecific and of a metabolic nature.

140. A Necrotizing Factor (NF) Isolated From Mouse Salivary Glands Exposed to the Salivary Gland Virus.—*Nicholas G. Grand, University of Illinois, Chicago.* When salivary gland virus (SGV) is injected subcutaneously into mice, intranuclear inclusion bodies appear in the salivary gland concomitant with a marked lymphocytic infiltration. A necrotizing factor can be isolated from the infected gland, using the following procedure: Pumice-ground SGV infected salivary glands are placed in an ether-acetone mixture, supernate removed, and ether and acetone is evaporated. The evaporated residue is suspended in Ringer's solution and stored at 4° C. This ether-acetone soluble fraction was shown to have a necrotizing effect on mouse skin. When the fraction is injected subcutaneously, a marked inflammatory reaction develops at the site of injection within a few hours and progresses to a deep necrotic ulcer in about 18 hours. Unlike the "menstrual toxin" or necrosin, which are thermolabile and destroyed when exposed to air, our necrotizing fraction (NF) is stable at temperatures of 75° C. and is not affected when exposed to air. Repeated injections of NF do not prevent the skin from becoming necrotic, which indicates that it does not provoke a strong immune reaction. The NF does not produce intranuclear inclusion bodies regardless of the route of introduction and will not protect a mouse from a lethal dose of SGV. Presently, we consider this necrotizing factor to be another fraction found in inflammatory exudate.

XIII. Enamel Solubility and Caries Susceptibility. March 22, Forenoon, Section A.

141. Changes in Microhardness of Enamel Following Treatment With Lactate Buffer.—*Ernest Newbrun, Philip Timberlake, and Ward Pigman, University of Alabama, Medical Center, Birmingham.* Previous studies of the in vitro development of carious lesions have indicated that for precise routine work, standard conditions need to be established if the efficiency of anticaries agents is to be evaluated. The rate of softening of enamel surfaces resulting from exposure to lactate buffers was investigated. Teeth were embedded in hard inlay wax contained in acrylic resin boxes, care being taken to keep the flattest portion lying horizontally. As no significant difference has been shown in the

hardness of various tooth types, anterior teeth were used, the hardness of the labial surface of these teeth being the most readily measured. The teeth were subjected for intervals up to 14 hours to a 10^{-3} N lactate buffer, pH 5.0 at 35° C. Using the Kentron hardness tester, 10 readings were taken of the hardness of each tooth surface before and after these treatments. Preliminary findings indicate that at the end of 2 hours the rate of decrease of hardness is slower than for longer times. This initial inhibition is probably attributable to the protection of the outer enamel layer.

142. Effect of Certain Acid Anions on the Solubility of Enamel and Dentin.—*Theodore Koulourides and Michael Buonocore, Eastman Dental Dispensary, Rochester, N. Y.* The dissolving capacity of a simple formic acid-sodium hydroxide pH 3.5 buffer of 0.1 M total acid concentration was determined by mechanically shaking 50 mg. of enamel and 68 mg. of dentin powders in 100 c.c. of the buffer for 15 minutes and analyzing the filtrate for dissolved Ca and P. The buffer was then retested after the separate addition of 0.01 M concentration of each of the following acids listed in order of decreasing strength, maleic, malonic, citric, malic, aspartic, succinic, and acetic. The pH in each case was readjusted to 3.5 with NaOH and remained constant during the reaction time. The results indicate that the addition of malic, aspartic, and citric acids to the formate buffer reduces its capacity to dissolve enamel by 11, 15, and 28 per cent, respectively. The other acids added did not change the enamel dissolving capacity of the formate buffer. With dentin, a reduction (15 per cent) was obtained only with the addition of citric acid. Maleic and malic acids each increased the dissolving capacity of the buffer 18 per cent. The differences in dissolution patterns of inorganically similar enamel and dentin, not explainable on ionic strength basis, suggests that their structural characteristics determine the extent and direction of specific anion effects on solubility.

143. Solubility of Enamel, Dentin, and Bone in Acetate, Lactate, Aspartate Buffers.—*Michael Buonocore, Eastman Dental Dispensary, Rochester, N. Y.* The dissolving capacities of simple pH 4 and 5 buffers of acetic, lactic, and aspartic acids of 0.1 M anion concentration was tested over 30 minutes against 50 mg. samples of powdered normal and anorganic enamel, dentin, and fresh defatted cortical human jaw bone. At each pH level, the lactate and acetate buffers showed similar dissolving capacities. The aspartate buffer showed over 50 per cent less dissolving capacity than the other buffers at each pH level. With 120-200 and 200-270 mesh dentin at pH 4, the dissolving capacities of the acetate and lactate buffers were approximately the same whereas, at pH 5, differences of the order of 10-20 per cent were evident depending on mesh size. At each pH level, the dentin-dissolving capacity of the aspartate was less than the other buffers with greater differences evident at pH 5. Dissolution patterns with bone of 120-200 and 200-270 mesh at pH 5 were comparable to those obtained with dentin. Generally the amount of normal and anorganic enamel dissolved at pH 4 was 2.5 to 3.5 times as great as at pH 5 whereas, with dentin, the ratio was between 1.5-2.2. The upper limit of ratios represent aspartate values. Results suggest existence of strong specific aspartate anion effects capable of attenuating the dissolving action of hydrogen ion toward enamel. Magnitude of specific effect appears in inverse relationship to substrate surface area and hydrogen ion concentration of dissolving medium.

144. Solubility Rate of Human Dental Enamel in Acid.—*J. A. Gray, The Procter & Gamble Company, Cincinnati.* Enamel solubility rate curves in

acid were determined by the differential and integral methods as a function of pH, temperature, agitation, and buffer concentration. The amount of enamel dissolved was measured by using radioactive teeth and determining the amount of activity appearing in solution. The reaction between acid and dental enamel was found to be diffusion controlled, being very dependent on agitation and only slightly dependent on temperature. In unbuffered solutions, the solubility rate was proportional to the hydrogen ion concentration. However, in buffered solutions, the rate was proportional to the concentration of the undissociated acid which supplies hydrogen ions to the reaction. Simple cations, when added to the acid solution, inhibited the dissolution of enamel while anions, in general, had no such effect. The accumulation of the reaction product, calcium ion, and changes in the hydrogen ion or undissociated acid concentration determined the solubility equilibrium reached between enamel and an acid solution.

145. Effects of Salivary Fluoride on Enamel Solubility.—*Wolfgang Buttner and Joseph C. Muhler, Indiana University, Bloomington.* Several authors have reported a wide range in the fluorine level of human salivas (5 μg to 74 μg F per 100 ml.), which seems to be independent of the amount of fluorine ingested in water and food. Because fluorine values in the saliva of caries-resistant individuals tended to be higher, it was assumed that salivary fluorine levels greater than 25 μg F per 100 ml. might inhibit caries. Laboratory evidence was obtained concerning the effect of salivary fluoride on enamel solubility. Sodium fluoride was administered at a level of 1 mg. F daily for 3 weeks to 3 groups of weanling rats either in the drinking water, by stomach tube, or intraperitoneally, respectively. In each group, one-half of the animals were desalivated. The acid solubility of intact molar teeth of the rat indicates a 25 per cent reduction of enamel solubility of the group receiving fluoride containing drinking water whether the rats were desalivated or not. If fluoride was provided by stomach tube or intraperitoneally, the reduction of enamel solubility ranged from 25 to 40 per cent in the nondesalivated rats. No significant reduction was found in the desalivated rats of these groups.

146. Identification of the Reaction Products of Stannous Fluoride and Sodium Fluoride With Powdered Dental Enamel.—*H. G. Langer and W. H. Nebergall, Indiana University, Bloomington.* Previously reported chemical studies have shown that the reaction which takes place under certain conditions between aqueous sodium fluoride and dental enamel is one of double decomposition. Calcium fluoride and sodium phosphate are formed, with etching of the enamel surface and liberation of phosphate to the solution. These observations were further substantiated; the reaction being studied by chemical analysis, infrared absorption, and x-ray diffraction. Similar investigations were conducted on the interaction of stannous fluoride with powdered dental enamel. The experiments were carried out under extreme conditions—high concentrations of SnF_2, elevated temperatures, and high pressures—to obtain extensive interaction and well-crystallized reaction products, which could be readily studied by infrared and x-ray technics. Synthetic hydroxyapatite was used in some experiments, principally because it was found to react more rapidly and completely than dental enamel, due to smaller particle size. In both cases, the reaction products were found to be calcium fluoride and an insoluble phosphate of stannous tin, the particular stannous phosphate formed depending upon the reaction conditions, such as concentration of SnF_2, temperature, pressure, and

particularly the pH of the aqueous slurry. It was also observed that essentially no calcium nor phosphate was liberated to the solution during the reaction of SnF_2 with dental enamel and hydroxyapatite.

147. The Effect of Enamel Morphology in Susceptibility to Bacterial Attack.—*Lee R. Brown, Jr., and Merrill G. Wheatcroft, U. S. Naval Dental School, National Naval Medical Center, Bethesda.* The purpose of this study was to determine whether morphologically defined areas in unerupted teeth, such as deep grooves, pits, fissures, and cloudy to opaque enamel, were more susceptible to bacterial attack than clear, smooth enamel. In order to establish areas where a concentrated bacterial attack might produce the appearance of a carious lesion, pin-point areas of enamel were isolated with wax. These teeth were subjected to bacterial cultures and sectioned for examination upon termination of the experiment. In order to permit the internal observation of the occlusal attack by the bacteria, teeth were sectioned into slices of approximately 250 microns and cemented between squares of glass to allow only the occlusal surfaces to be exposed to the cultures. Both test tube and continuous culture technics were used, employing 4 types of cultures—*Streptococcus salivarius, Lactobacillus casei,* a mixture of proteolytic organisms, and a saliva inoculum. It was observed that where the effects of acid production predominated, either in mixed or pure cultures, a general rather than a specific attack on the teeth was noted. No selectivity was observed with respect to the morphology of the tooth. After 14 days of exposure, the enamel was dissolved evenly over the entire surface.

148. Chemical and Morphologic Factors in Susceptibility of Enamel to Acid Attack.—*L. W. Wachtel, U. S. Naval Dental School, National Naval Medical Center, Bethesda.* The significance of the varied organic content of enamel with respect to surface acid attack (0.01 N nitric or 0.07 N lactic acid) was studied, utilizing tooth slices of 200 microns thickness protected on the cut sides by cemented glass. No differences could be observed in the speed and severity of attack between sections of enamel containing relatively high amounts of organic matter (0.4 per cent nitrogen) and those with low amounts (0.07 per cent nitrogen). No selectivity was evidenced for deep fissures; the acid dissolved high areas of the tooth slice first, and then wore down the remainder of the tooth evenly. Artificially prepared pin-point sites on the surface exhibited a cavitation type of erosion, radiating equally in all directions; the deep fingerlike attacks noted in normal caries were not observed. Analysis of resulting solutions indicated that the enamel was solubilized by conversion to the diacid phosphate salt (H_2PO_4), 5 ml. of 0.01 N nitric acid dissolved 3.5 ± 0.6 mg. of tooth. These studies support the opinion that mechanisms other than acid production in the mouth are involved in the production of typical dental caries.

149. Caries of Enamel.—*Dimitri Afonsky, The Colgate-Palmolive Company in cooperation with Rutgers University, New Brunswick, N. J.* Caries of the enamel was studied in ground and decalcified sections of teeth obtained from mice, Mongolian gerbils, and man. There was a marked similarity in the development of the lesions. The earliest manifestation of caries was brown pigmentation of the enamel, which might extend through its thickness. The most striking observation was the changes in the organic matrix. While normal enamel was almost completely destroyed in decalcified sections, in cases of caries there invariably remained organic matrix. As the enamel involvement progressed, the amount of the matrix proportionately increased. In its structure and amount, the matrix was similar to that present during developmental

stages of the enamel formation. The whole thickness of the enamel might thus be involved without any evidence of its destruction or invasion by microorganisms. Occasionally, the matrix was observed to assume a homogenous character prior to its destruction. The invasion by microorganisms is a late process. This may take place in several locations at once, and the presence of lamellae is not essential. Observations indicate that caries of the enamel cannot be adequately explained by decalcification of its inorganic portion nor by proteolysis of its organic matrix. Rather, certain changes take place in the matrix which result in its increase to the amount present in immature teeth.

150. The Use of Vital Stains as Indicators for Beginning Caries.—*Robert M. Stephan, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* The development of carious lesions in the incisor teeth of rats has suggested the use of vital staining to detect the earliest stages of caries, and to serve as an indicator for factors controlling caries susceptibility. Thirty-eight stains and colorimetric indicators have been tested using Osborne-Mendel strain rats from the N.I.H. animal production section. The rats were fed Diet 593 consisting of 83 per cent sucrose, 12 per cent casein, 4 per cent H.M.W. salt mixture, and 1 per cent mixture of essential vitamins, and the drinking water contained 10 per cent of added glucose. In different experiments, the vital stains have been (1) injected intraperitoneally, (2) mixed with food or drinking water, or (3) applied directly to the teeth during observation under the stereomicroscope. These procedures permit the staining of tooth substance before, during, or after the teeth have been exposed to caries attack in the oral environment. In some cases where tooth substance had been vitally stained during its formation, the stain was subsequently removed in areas of localized caries development. Where vital stains were present in food or water during the period of caries development, staining generally occurred selectively in and around the lesion. The addition to the drinking water of 0.01 per cent of certain anthraquinone dyes, such as alizarin red S and alizarin blue S, furnished good stains for detecting early caries. Most of the dyes tested which had other types of chemical structures, proved ineffective for this purpose.

151. Quantitive Assessment of Radiodensity and Birefringence in Incipient Carious Lesions.—*Narendar Soni, Finn Brudevold, and Ernest Newbrun, Eastman Dental Dispensary, Rochester, N. Y.* Ground sections of incipient caries with outer enamel appearing intact were studied microradiographically and under polarized light. The microradiographs were assessed with a microdensitometer capable of measuring the optical densities of areas as small as 200 square micron. The optical density was plotted by an automatic recorder as the logarithm of the intensity of the transmitted light. Birefringence was measured with a polarizing microscope. Normal enamel showed maximal radio density in the outermost enamel and a gradual decrease toward the dentin which was most pronounced in the outer one fourth and in the innermost portions of the enamel. Total decrease in density averaged approximately 10 per cent. In incipient caries the reduction in density of the outermost enamel averaged 30 per cent, demonstrating partial decalcification. Structural details including enamel prisms were visible in demineralized areas which had reduced radiodensity of 20 or more per cent. Variations in densitometric readings within the lesion corresponding to Retzius' striae could be seen. With present technics, densitometric readings within different areas of carious lesions could be only partly correlated with zones of different birefringence. Microradiographic reproduction of structural details within lesions depend more on the time of exposure than on orientation of the section in relation to the x-ray beam.

XIV. Periodontium. March 22, Forenoon, Section B.

152. Observations on Some Genetic Characteristics of the Periodontium in Mice.—*Paul N. Baer, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* Mice of strains, DBA-2JN, C57L, and STR/N were fed the following diets: (1) Purina Fox Chow, of a hard physical consistency, (2) a high fat diet of a soft physical consistency, (3) a high fat diet with a cellulose filler, also of a soft physical consistency, and (4) a nutritious diet of watery consistency. A total of 150 mice have been observed to date. At the time of sacrifice the animals were 16 months of age. Seventy-two animals, 24 from each strain, were studied histologically, and the remainder were defleshed and studied grossly. The results to date are as follows: (a) The trabecular pattern of the alveolar bone is distinct for each strain regardless of the nature of the diet. (b) Strain STR/N is susceptible to periodontal disease, while the other 2 strains are more resistant. (c) In strain DBA-2JN, the maxillas of the animals on the high fat and high fat plus filler diets are wider from first molar to first molar than those on stock diet. These differences are statistically significant. (d) In strain C57L, the mandibles of the animals on the high fat and high fat plus filler diets weigh more than those on stock diet. These differences are statistically significant. (e) The physical consistency of the diet does not seem to be as important as formerly stated in causing wear patterns on teeth or as an etiologic factor in periodontal disease. (f) The mandibles in 14 per cent of the C57L strain were fused at the symphysis. This was an interesting finding that has not been observed to occur in animals of any other strain studied so far.

153. The Organization of the Periodontal Membrane Fibers of Developing Molars.—*Sol Bernick, University of Southern California, Los Angeles.* Young rats, 15 to 35 days old, were used to study the formation and organization of the periodontal membrane fibers of the developing molars. The teeth and jaws were fixed in an alcoholic Bouin's solution. The specimens were decalcified, dehydrated, and infiltrated for nitrocellulose embedding in the routine manner. The embedded blocks of tissue were sectioned at 20 to 50 micra thicknesses, and alternate cut sections were then stained with hematoxylin and triosin, Mallory's connective tissue stain, Masson's trichrome, periodic acid-Schiff method, and Pearson's silver impregnation technic. In the early development of the periodontal membrane, there is a definite oblique orientation toward the gingival region of the fibroblasts. At the same time the attachment fibers are observed arising from the cementum paralleling the direction of the fibroblasts. These fibers are entrapped in the forming alveolar bone. As the root continues in formation the cells as well as the fibers maintain this fundamental relationship. As the tooth erupts toward the oral mucosa the fibers become organized into the functional 6 groups described in the classical manner.

154. The Effect of Vitamin A Deficiency in Periodontal Structures of Rat Molars.—*Duni C. Miglani, University of Texas, Dental Branch, and M. D. Anderson Hospital, Houston, and H. A. Zander, Eastman Clinic, Rochester, N. Y.* Albino rats, 40 days old, were kept on a vitamin A deficient diet for a period of 13 weeks. The periodontal tissues of these animals were compared histologically with those of control animals fed on the same diet supplemented with vitamin A. A great emphasis was made on the areas of cementum resorption. The histologic examination revealed hyperkeratosis and hyperplasia of the gingival tissue, atrophy of alveolar bone, calcification and irregular narrowing of periodontal membrane, excessive cementum resorption, and ankylosis of teeth. The difference in the amount of cementum resorption in the 2 groups is of statistical significance.

155. COMPARATIVE GERONTOLOGY OF THE PERIODONTIUM OF THE RAT AND HAMSTER.—*J. Klingsberg and E. O. Butcher, New York University College of Dentistry, New York.* Sections of the jaws of rats and hamsters, 7 to 700 days old, were stained with hematoxylin and eosin, Mallory's trichrome, Van Gieson's picrofuchsin, or chlorantine fast red. At all ages the periodontal membrane of the hamster tends to be more variable in width, thinner and more vascular than that of the rat. Only in aged rats are areas of comparable thinness found. At the age of 700 days, the alveolar bone and marrow are partly replaced by fibrotic areas in which there is an increase of fibroblasts. Both immature animals have numerous nuclei in all regions. The nuclei of the immature rat tends to be flatter and the collagenous fibers finer and more regular in arrangement. In the aged animals, all shapes of fibroblastic nuclei can be found. As the animals age, cellularity diminishes and fibers increase in density. At 500 days the tissue assumes the appearance of tendon in the alveolar crest and transeptal regions. The thickness of the epithelium of the attached gingiva does not change significantly up to 700 days. The keratinized layer increases in thickness to 300 days. There is an increase in the rete ridges to 450 days, followed by an apparent decrease around the six hundred and fiftieth day. A gradual recession of the epithelial attachment occurs in both animals without pocket formation.

156. BIOCHEMICAL AND METABOLIC DETERMINANTS OF EXPERIMENTAL GINGIVAL INFECTIONS.—*Philip Person and Albert Fine, VA Hospital, Brooklyn.* The biochemical and metabolic status of experimental animals and their oral tissues are rarely considered during attempts to transmit periodontal infections from human or other sources. In the present experiments, host metabolic processes were studied as possible determinants of gingival lesion production. The metabolism of guinea pigs was altered by intraperitoneal injections of fluoracetate, malonate, citrate, and also by starvation. Gingival crevice scrapings from human mouths with varying degrees of gingivitis were homogenized in saline and injected subgingivally in the above and in control animals. The following results were obtained: (1) The sequence of tissue changes when lesions were produced by subgingival injection of homogenates was edema, redness, local abscess formation, and healing. Cellulitis was also seen in some animals. (2) Gingival lesions were produced in some nonmetabolically disturbed animals, but, (3) in fluoracetate-treated or starved animals, lesions developed sooner, were more severe, and lasted longer than in any of the other groups. (4) if, as soon as lesions in fluoracetate-treated or starved animals healed, the animals were rechallenged with fluoracetate, a recurrence of the lesion was provoked. Such recurrences could not be provoked in control, malonate, or citrate-treated animals. The results suggest that intracellular biochemical and metabolic processes may act as important determinants of the onset and course of microbial and other involvements in periodontal disease.

157. A NEW STAIN FOR CONNECTIVE TISSUES OF THE PERIODONTIUM.—*Harold M. Fullmer, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* Tendons, ligaments, and periodontal membranes are believed to consist almost entirely of collagen fibers. When sections of these tissues are subjected to the staining procedure given below, some fibers, previously described as collagen, react with the purple stain, while others remain unreactive and are colored by the counterstain. Connective tissues subjected to stress apparently develop special fibers demonstrated by this stain. The stain can be used after formalin or alcoholic fixatives, and is performed as follows: (1) Deparaffinize and bring sections to water. (2) Oxidize for 30 min. 25° C. with peracetic or performic acid. (3) Wash in running water. (4)

Stain with aldehyde fuchsin for 5-10 min. (5) Differentiate in 95 per cent ethanol for 15 min. (3 changes of 5 min. each). (6) Stain in Lillie-Mayer hematoxylin 4 min. (7) Rinse in distilled water, differentiate in 0.5 per cent HCl/95 per cent ethanol for 3-4 dips, and blue in running water for 5-10 min. (8) Counterstain in the following solution advocated by Halmi for 20 sec.: distilled water, 100 ml.; light green SF yellowish, 0.2 g.; orange G, 1.0 g.; chromotrope 2R, 0.5 g.; phosphotungstic acid, 0.5 g.; and glacial acetic acid, 0.1 ml. (9) Rinse in 0.2 per cent acetic acid in 95 per cent ethanol. (10) Dehydrate in absolute alcohol, clear in xylol, and mount. Bone, dentin, and scirrhous connective tissues are stained yellow; collagen and epithelial cells, green to greenish yellow; nuclei, yellowish brown; mast cell granules, acid mucopolysaccharides, and keratin, purple. In addition, numerous connective tissue fibers are found that stain a brilliant purple. In the periodontium these fibers frequently take the course of the periodontal fibers and are inserted into the cementum and alveolar bones; others, particularly in developing teeth, run in an occlusal-apical direction.

158. Correlation of Blood Constituents With Periodontal Condition.—*D. Y. Burrill, A. Doner, and L. S. Fosdick, Northwestern University Dental School, Chicago.* One hundred fifty subjects whose radiographs showed little or no loss of periodontal bone, and 70 whose radiographs showed severe loss of periodontal bone each donated 25 c.c. of fasting blood. For each, a determination was made of hemoglobin, erythrocyte count, catalase, acid and alkaline phosphatase, aliesterase, acetylase, and choline esterase. Except for the choline esterase there were no consistent differences between the values for the patients with good periodontal bone and those for patients who had suffered severe bone loss. Choline esterase was generally higher in the blood of subjects with marked periodontal disease than in the blood of normals. The mean choline esterase values were, for the normals, 50 units per ml., and for the periodontally involved 57 units per ml.

159. Hydrolytic Enzyme Sources and Periodontal Disease.—*V. F. Lisanti, Tufts University School of Dental Medicine, Boston.* Continued studies on the relationship of certain hydrolytic enzymes and gingival disturbances have shown that β-D-glactosidase and β-D-glucosidase are of bacterial origin. β-D-Glucuronidase has been identified as having both mammalian and bacterial sources. When these enzymes are found in diseased areas, it is now possible to differentiate histochemically between bacterial and mammalian sources. Normal gingival areas do not demonstrate enzymes of bacterial sources. These bacterial enzymes have been found in the following areas of diseased gingiva: the desquamating keratinized surface of the epithelium, the epithelial attachment areas, the entire soft tissue aspect of the periodontal pocket, the deeper layers of both connective tissue and epithelial tissue leading from the abnormal areas. Quantitative assays performed upon the aforementioned diseased tissue areas have shown dramatic increases in bacterial enzyme.

160. Effects of Enzymes on Fixed Human Gingivae.—*Wm. J. Carter, University of Kansas City, School of Dentistry, Kansas City, Mo.* Previous investigations have demonstrated enzymic cytolysis of epithelium by filtrates of feces from patients with ulcerative colitis. This work has been duplicated and extended to include filtrates of subgingival exudates from periodontal patients. Gingival tissue was "formalin fixed" and incubated with filtrates (B.D. Swinney filter) of infected materia alba and plaque. Heat-treated filtrates were used for controls. Fixed gingival tissue was similarly treated with known enzymes: trypsin, pepsin, and papain as reference material. The only gross changes observed so far in fixed tissue specimens were those subjected to ulcerative colitis

fecal filtrates and the known enzymes. There have been minor cytologic changes in ginigval tissue treated with periodontal pocket filtrate. This work is being continued on sterile fresh ginigval tissue and in tissue culture.

161. A Histochemical Study of the Gingiva of Human Beings in Pregnancy.—*Samuel S. Turesky, Beverly Fisher, and Irving Glickman, Tufts University School of Dental Medicine, Boston.* Gingival biopsies of 30 pregnant patients were studied histochemically. Female patients of comparable ages were used as controls. Each specimen consisted of marginal and attached gingiva and areolar mucosa. Serial sections of each specimen were stained as follows: hematoxylin and eosin; Barrnett-Seligman technic for protein-bound sulfhydryl and disulfide groups; Weiss, Tsou, Seligman technic for alpha amino groups; Lillie's periodic acid-Schiff technic for glycogen and other polysaccharides. In both the pregnant and nonpregnant individuals the appearance of the marginal gingiva was complicated by the presence of inflammation. In the attached noninflamed gingiva the following features were noted in the pregnant patients: A more pronounced tendency toward the elongation of the rete pegs with concentration of cytoplasmic glycogen, protein-bound amino groups, sulfhydryl and disulfide groups, a thinning of the basement membrane, a reduction or absence of water insoluble and alcohol insoluble PAS positive substances in the papillary layer of the connective tissue. There was less surface keratinization and more parakeratinization in the pregnant subjects.

162. Gingival Hyperplasia—A Study of the Effect of Anticonvulsant Drugs Upon Epileptic and Mentally Retarded Patients.—*Harold J. Panuska and Robert J. Gorlin, School of Dentistry, University of Minnesota, Minneapolis.* An oral survey of 1,048 patients was performed at Cambridge State School and Hospital to determine the effects of anticonvulsant drugs upon the oral mucosa. The patients were epileptic or mentally retarded. Several drugs such as Dilantin sodium, phenobarbital, Mebaral, Mesantoin, various bromides, Hibicon, etc., were used alone or in combination. An attempt was made to determine whether any potentiation or diminution of Dilantin hyperplasia would result when these drugs were used in combination with Dilantin. Results indicated that patients receiving phenobarbital alone showed some hyperplasia but when used in combination with Dilantin the hyperplasia associated with phenobarbital was masked to a degree of insignificance. The other anticonvulsants did not show any potentiation of hyperplasia associated with Dilantin. A correlation was observed among Dilantin, Hyperplasia Index, and oral hygiene; as the oral hygiene became poorer and the Dilantin dosage greater, the Hyperplasia Index increased. The length of time patients were treated with Dilantin was not clearly correlated with the degree of hyperplasia. The data only reflected a difference in sample size and no clear-cut positive correlation could be ascertained. Dilantin hyperplasia had similar effects on both sexes, but was somewhat more common in males. Conclusion could not be drawn concerning racial differences. The presence of excessive hair (hirsutism), suggested by Keyes and Esterberg, could not be substantiated.

163. The Development of an Instrument to Measure Tooth Mobility.—*Gilbert J. Parfitt, University of Alabama School of Dentistry, Birmingham.* The tissues that hold the teeth in their sockets provide both cushioning and tensile strength. Among the properties of interest are tensile strength, elastic range, compressive strength, fluid content, and response to transient forces. It is known that some of these properties vary with physiologic changes. The immediate aim of this project was to develop an instrument with sufficient sensitivity to measure the slight alterations in deflection of a tooth under loads up

to 5 pounds, applied in a reproducible direction. The ultimate aim was to investigate the effect of systemic and local conditions on the tooth-supporting structures by utilizing this method of measurement. Linear variable differential transformers were used to measure the deflection of a tooth relative to adjacent teeth and to measure the applied force. A Moseley X-Y Recorder was used to record the differential secondary voltages from each transformer. Several complete calibrations and measurements have been recorded with a trial model. Mobilities of 75 to 105 micro-inches per pound have been recorded clinically on upper incisor teeth. The development of a clinical model is continuing.

XV. Pulp. March 22, Forenoon, Section C.

164. Comparison of Bovine, Sheep, and Human Dental Pulp Oxygen Quotients.—*Peter R. Boeke, Alton K. Fisher, Daniel J. Spinella, and Edward M. Braley, School of Dentistry, University of Iowa, Iowa City.* Previously, the level of oxygen consumption in bovine dental pulp was related to the state of tooth development. A mean QO_2 of 2.04 was observed in teeth showing no root formation. A progressive decrease in oxygen consumption was noted as root formation advanced, reaching its lower mean level of QO_2 0.47 when the roots were fully formed. In the present study, pulps from sheep teeth in various stages of development showed mean QO_2 values that were very similar to those of bovine pulp. The mean first hour QO_2 determinations of tissue from teeth with no root formation was 2.35, that of teeth with incipient root formation was 0.98, and that of teeth with fully formed roots was 0.36. Similar studies on human dental pulp, while very incomplete, show less consistency in relation of the QO_2 to the state of tooth development. While the rate of oxygen consumption in one fully formed tooth was 0.57 microliter per milligram of dried tissue, another fully formed third molar pulp from a 27-year-old man showed a QO_2 of 3.33. The mean QO_2 of bovine dental pulp, irrespective of the state of tooth development based on 885 determinations, is 0.73. The mean QO_2 of human dental pulp, based on 25 determinations, is 1.40. The rate of oxygen consumption in human pulp observed in this study is considerably higher than the value of 0.31 reported by Pincus; whether the difference between bovine and sheep and human tissue respiratory rates is attributable to peculiarities of the basal metabolism of the various species, or to differences in the complexity of psychogenic stimuli associated with slaughter or surgery that effect metabolism, has not been determined.

165. A Qualitative Chromatographic Analysis of Amino Acids of Bovine Dental Pulp.—*Daniel J. Spinella and Alton K. Fisher, College of Dentistry, State University of Iowa, Iowa City.* Studies of amino acids of the dental pulp are in progress to determine the qualitative and quantitative relationship of these constituents to those present in dentin. The preliminary phase of this work has consisted of a qualitative analysis of bovine dental pulp by descending one dimensional paper chromatography. Hydrolyzates were prepared according to the recommendations of Block, and preparation of final sample solutions and pH adjustment of the solvents and papers was done according to the recommendations of McFarren. Filter paper sheets, approximately 56 by 46 cm., were employed in order to permit application of several spots of the unknown sample and also of a known purified amino acid on the same sheet. Identification of the various amino acids detected in hydrolyzates of pulp tissue was made both by comparison of the developed spots of the known and unknown samples and by calculation of r. f. values. The amino acids tentatively identified in this tissue are alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, valine, phenylalanine,

proline, serine, threonine, tryptophan, and tyrosine. These results are similar to those reported for dentin by other workers. The greatest recovery of nitrogenous material in the purified hydrolyzates prepared by acid hydrolysis was accomplished by digestion for at least 24 hours and not longer than 30 hours. Within this range the nitrogen yield was approximately 82 per cent of that in the original material.

166. Protein Fractions in Dental Pulp Fluid and in Blood Plasma.—*George Steelmon, John Haldi, and Winfrey Wynn, Emory University, Emory University, Ga.* Dental pulp fluid was obtained from the teeth of anesthetized dogs through a small polyethylene tube inserted into a hole drilled by the Cavitron through the enamel and dentin to the pulp chamber. Determinations have been made by paper chromatography and electrophoresis on the albumin and globulin fractions of the dental pulp fluid and the blood plasma of the same animals. The resultant curves obtained from a self-recording densitometer from the dental pulp fluid and the blood plasma (in different experiments) will be presented together with planimetric measurements of the areas under the curves. The protein content was invariably higher in the plasma than in the dental pulp fluid.

167. Pulp Healing Following Capping in Human Sound and Carious Teeth.—*Harold Berk, Tufts University, School of Dental Medicine, Boston, and Harold R. Stanley, Jr., National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* The maturation rates of the callus formation due to a pulp-capping procedure in human sound teeth with open roots and carious teeth with closed roots were compared histologically. A calcium hydroxide methyl cellulose paste was employed. Each group consisted of 9 teeth. Hematoxylin and eosin, Masson's trichrome, and Wilder's reticulum stains, and the periodic acid-Schiff reaction were used. The specimens were compared on the basis of the most differentiated portion of the callus adjacent to the vital pulp. The usual, well-defined necrotic zone and line of demarcation, resulting from the application of the pulp capping material, were observed. The most mature portion of the callus was recorded as: fibrous, consisting of argyrophilic fibers; secondary irregular predentin or dentin, with few if any tubules; or secondary tubular predentin or dentin. Secondary tubular predentin was recognized as indicating resolution of the pulpal lesion with a favorable prognosis. The maturation rate of the callus required less time in the young sound teeth than in the older carious teeth. Tubular predentin formation could be observed as early as 2 weeks in the sound teeth. The older teeth required a prolonged production of secondary irregular predentin or dentin. Only a minimal amount of secondary tubular predentin was found at 12 weeks in the older carious teeth. With adequate time, the same degreee of maturation was found in both groups.

168. Tissue Changes Under Acrylic and Porcelain Pontics.—*John R. Allison, and H. L. Bhatia, Loyola University Dental School, Chicago.* Acrylic pontics are being widely used in fixed dental prostheses. Whereas, a large number of studies have been conducted to investigate the effects of acrylic fillings on the dental pulp, few studies have been done on its effects on the oral mucous membrane. This study was conducted on adult Rhesus monkeys with permanent dentition to study and compare the histologic tissue changes under acrylic and porcelain (glazed and unglazed) pontics. Extractions were performed on all permanent first molars on adult monkeys and the tissues were allowed to heal for a period of 6 weeks. Fixed bridges were constructed replacing the first molars with pontics of acrylic, glazed porcelain and unglazed porcelain. Full gold crowns were used as abutments. The bridges were cemented in place for

a period of 3 months. The animals were sacrificed and histologic slides were made of the tissues underlying each pontic. Little or slight inflammatory changes were found under glazed porcelain as compared with extensive inflammatory changes under acrylic.

169. Bacterial Localization in Pulps of Rat Molars Following Bacteremias.—*George W. Burke, Jr., and Holmes T. Knighton, Medical College of Virginia, Richmond.* Cavities prepared in maxillary first molars were filled with cement or eugenol and zinc oxide. Croton oil was applied to some cavities before filling. Animals were injected intravenously with standard amounts of *Staphylococcus aureus* 15 minutes later and tail blood obtained to detect bacteremias. After 1 or 7 days, heart blood was obtained and the animals were sacrificed. Maxillas were removed and pulpal tissue was obtained from treated first molars and second molar controls. Blood and pulpal tissues were cultured in broth and subcultured on a selective medium to detect *S. aureus.* In 96 animals with positive bacteremias following injections, but negative at date of sacrifice, the incidence of *Staphylococcus aureus* was as follows: (1) 2 of 192 pulps in untreated teeth were positive; (2) in animals sacrificed 1 day after injections, 17 of 46 nonexposed and 30 of 52 exposed pulps were positive and application of croton oil before filling cavities did not seem to influence results; and (3) in animals sacrificed 7 days after injections, nonexposed pulps were all negative. However, the following exposed pulps were positive; 3 of 20 under eugenol and zinc oxide, 4 of 14 under cement, and 17 of 30 treated with croton oil before filling with cement. To investigate possibilities of contaminations, 20 first molar pulps were exposed and cavities filled with cement. Animals were sacrificed 1 day later without receiving bacterial injections. Cultures from these pulps were uniformly negative for *S. aureus.*

170. Bacteriologic Status of Periapical Tissue in 150 Cases of Infected Pulpless Teeth.—*Louis I. Grossman, University of Pennsylvania, School of Dentistry, Philadelphia.* Four methods are available for determining bacteriologic status of periapical tissue, namely, (1) via root canal, (2) via root surface of extracted tooth, (3) via trocar and cannula, and (4) via root resection. Material in this study was obtained from periapical tissue of pulpless teeth with relatively large areas of rarefaction during root resection. Control cultures of the mucosa and labial plate of bone were taken prior to cultures of root apex, adherent soft tissue, and osseous bed. Data in 41 cases had to be discarded either because controls were positive or because a complete set of 5 cultures were not available. Of 109 cases, all cultures—2 of controls and 3 of periapical tissues—were negative in 93 (85.3 per cent) cases.

171. The Biology of High Speed Rotary Operative Dental Procedures: 1. Correlation of Tooth Volume Removed and Pulpal Pathology.—*Leo Zach, N. Y. U. Dental College, and Gerson Cohen, Veteran's Administration Hospital, New York.* In the course of a continuing study which aims at a bioassay on new high-speed (100,000 r.p.m. or better) operative instruments, certain variables must be studied in order to determine the effect of each of the physical components which might induce pulp pathosis. The preliminary study of one of these variables constitutes the basis of this report. Two immature Rhesus monkeys with mixed dentitions received, after Nembutal i.m. anesthesia, a total of 36 cavity preparations in 3 quadrants; with one quadrant unoperated as control. Preparations varied widely in depth, outline form, and total enamel and dentin removed. The instruments were a Page-Chayes handpiece, water-spray cooled, with a No. 36 carbide-bur rotating at 140,000 r.p.m. An attempt was made to achieve ranges of cavity bulk extending from merest pit to deep,

enclosing type of preparation, such as M.O.D.'s on molars. Preparations were filled with inlay wax of known specific gravity, removed, dried and weighed to the nearest milligram as an indication of bulk removal. Animals were sacrificed after 30 days and sections were cut en bloc at 7 micra. Specimens revealed a distinct, almost linear correlation between bulk of dentin removed and pulpal reaction. Secondary dentin stimulation was most pronounced, as was coarsening of the pulpal stroma, in the more extensive preparations. An unusual degenerative response of odontoblasts on opposing pulpal walls (opposite to the area of directly cut tubules) was noted. A possible cause is postulated in harmonic vibrations induced by extremely high rotary speeds.

172. Human Pulpal Reactions Following 20,000 and 150,000 R.P.M. Cavity Preparations.—*Herbert Swerdlow and Harold R. Stanley, Jr., National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* Typical Class V cavity preparations were cut on the facial surfaces of 113 noncarious, vital human teeth. The patients' ages ranged from 27 to 57 years (average 40.7). A conventional ball-bearing handpiece rotating an inverted cone No. 37 diamond stone with and without water spray was utilized for the 20,000 r.p.m. category. A belt-driven, rubber chucked, contra-angle handpiece rotating an inverted cone No. 35 tungsten carbide bur at 150,000 r.p.m. was employed with water spray. The teeth were air dried, filled with zinc oxide eugenol and extracted at intervals from one half an hour to 132 days. The three operative techniques were compared on the incidence of "questionable prognosis." This term includes teeth with intrapulpal abscesses or specimens with delayed healing, characterized by foci of chronically inflamed granulation tissue with persistent infiltrations of leukocytes. The 150,000 r.p.m. technique using the slightly smaller bur was obviously less traumatic than 20,000 r.p.m. Although the average thickness of remaining dentin was initially less in the 150,000 r.p.m. category and the remaining dentin of the specimens in this group with a questionable prognosis lesion was less than half of the other two categories, this procedure still developed the smallest percentage of lesion. The groups of teeth in this study suggest a milder pulpal reaction at 150,000 r.p.m. than at 20,000 r.p.m. The advantage of the water spray coolant in removing tooth structure is conclusively demonstrated.

173. Microcinematography of the Living Dental Pulp. II. Experimental Results.—*Mauri Pohto and Arje Scheinin, Institute of Dentistry, University of Helsinki, Finland.* A film shows the effect of hydrogen peroxide and ethyl alcohol solutions on the vascular bed in the incisors of living rats. In the exposed pulp, a 3 per cent solution of hydrogen peroxide was found to penetrate the walls of the blood vessels causing intravascular emboli and severe damage to the vascular bed. In the unexposed pulp, a 3 per cent solution of hydrogen peroxide was found to penetrate the dentinal wall and to form intrapulpal emboli. In these cases the thickness of the dentinal layer covering the pulp varied between 20 to 50 microns. The pressure of the embolisms exceeded the blood pressure and stopped the flow temporarily. Ruptures of the walls of the vessels occurred and caused hemorrhages. The effect of ethyl alcohol solutions at different concentrations was similarly studied in the exposed and unexposed dental pulp. In the exposed pulp, 40 and 70 per cent solutions applied for 10 seconds stopped the flow in many of the vessels. In the unexposed pulp, where the thickness of the dentinal layer covering the pulp was 40 microns, the flow was temporarily stopped in the superficial vessels.

174. Microcinematography of the Living Dental Pulp. III. Experimental Results.—*Mauri Pohto and Arje Scheinin, Institute of Dentistry, University of Helsinki, Finland.* A film shows the effect of cold and heat on the

vascular bed in the incisors of living rats. In the cases presented, most of the controls are perfused with 0.5 per cent gelatin-containing Ringer solution at 37° C. In the cold experiments, the temperature of the perfusing solution is lowered to 3° C. This causes a marked retardation of the blood flow in the exposed pulp. In the unexposed pulp the temperature is lowered to 31° and 24° C., with the same result. Reversal of temperature to 37° C. restores original conditions. In the heat experiments, a rise of temperature of the perfusing solution to 39° to 40° C. causes hyperemy and accelerates the flow. Stasis and thrombosis occur in the exposed pulp at 46° to 50° C. In these cases the exposure to heat was maintained for 30 seconds. In the unexposed pulp, where the thickness of the dentin covering the pulp was 25 to 80 microns, slightly higher temperatures were tolerated. Final thrombosis occurred at 56° to 60° C.

XVII. Fluorides. March 22, Afternoon, Section A.

175. The Half-Life of Radioactive Fluoride.—*W. D. Armstrong, Leon Singer, and Curtis Carlson, University of Minnesota, Minneapolis.* Radioactive fluoride (F^{18}) will find a greater use in dental research through improved methods of production and of characterization of its properties. Water enriched to 3 per cent abundance with O^{18} is enclosed in conveniently prepared polyethylene cells, which will be described, and bombarded with 10 MEV protons produced by a linear accelerator. Large yields of carrier free F^{18} are produced. A short-lived (10 min.) radioactive contaminant, presumed to be N^{13}, can be removed if required by distillation for perchloric acid. The isotope so isolated decays at a constant rate. Observations made over a period of 10 hours, and analyzed by a method of least squares, indicate that the half-life of F^{18} is 109.3 min. as compared to the literature value of 112 min. The use of the newly determined half-life of F^{18} gives excellent agreement of results obtained over long periods of time in contrast with those calculated through the use of 112 min. as the half-life.

176. Analysis of Fluoride in Plasma and Serum.—*Leon Singer, W. D. Armstrong, P. Venkateswarlu, Jeanne Sheck, and Norma Cummins, University of Minnesota, Minneapolis.* A micro method for fluoride analysis has been described. A procedure for the analysis of fluoride in 5-10 ml. of plasma or serum has been developed and will be described and the results of analyses to demonstrate the reliability of the method will be reported. Bovine serum from 5 different animals has been repeatedly analyzed. The average values of the individual animals' sera varied between 0.12 and 0.27 ppm. The plasma of two human subjects on repeated analyses has given average values of 0.12 and 0.13 ppm fluoride. Pooled dog serum contained 0.21 ppm fluoride.

177. Lack of Effect of Fluoride on Urinary Calculi Formation.—*P. Venkateswarlu, Leon Singer, and W. D. Armstrong, University of Minnesota, Minneapolis.* An investigation of the effect of fluoride ingestion on the incidence of urinary calculi in the rat has been completed. One hundred young rats, divided into two equal groups, were maintained on McCarison's urinary calculi-forming diet. Group I, the control group, received de-ionized distilled water and Group II received the same water to which fluoride was added to a level of 10 ppm. All animals were examined post-mortem for the presence of calculi in the urinary tract. The chemical analyses of the stones will be reported later. In the urinary tracts of the animals of Group I, there were 13 animals with definite urinary stones, 4 with fine crystals mixed with organic matter, and 3 with a soft curdy material not usually met with in the normal urinary tract. In Group II there were 9 animals with definite urinary stones, 3 with the fine

crystals in organic matter, and 5 with the soft curdy material. No difference in the longevity of the animals was noted and the incidence of edema of feet and tail, abscesses of the tongue, gastrointestinal disorders, gangrene of the tail, etc., have been recorded. There was no evidence to suggest that fluoride ingestion via drinking water causes an increased incidence of urinary calculi in the rat when the fluoride supplement was superimposed on a regimen causing the formation of urinary calculi.

178. Dietary Utilization of Fluorine in Presence of Calcium Salts.—*Robert E. Radcliff, Louis B. Spear, Jr., and Joseph C. Muhler, Indiana University, Indianapolis.* Previous studies have suggested that added dietary fluoride (25 ppm F) is less efficiently retained in the skeleton of the rat in the presence of soluble calcium salts than in the absence of calcium. When no fluoride is added to the diet (the fluorine present only as a contaminate to the dietary constituents; 2-4 ppm F), this effect is not observed. When the calcium and phosphorus content of the basal ration was increased tenfold by the addition of $CaCO_3$ and NaH_2PO_4, a pronounced failure of the rats to gain weight normally occurred. This latter effect may result from improper dietary relationship of Ca:P, or to inadequate vitamin D. In order to extend these findings, 80 weanling rats were divided evenly into 8 groups according to food consumption which was previously determined during a 5-day period. All animals in the 8 groups were pair fed. Group I received a stock corn diet; Groups II, III, and IV, the same diet except to diet II was added 5 times the calcium and phosphorus in the basal diet and to Diet III 10 times. Diet IV contained 25 ppm F. Diets V and VI received the stock diet plus 25 ppm F and, in addition, Group V 5 times the dietary Ca:P level, and Group VI 10 times the amount. Group VII received 10 times the Ca:P level and 850 U.S.P. units of vitamin D. Group VIII received 10 times the Ca:P level, fluoride and vitamin D. The results indicated that the addition of vitamin D did not prevent the weight loss. The data also confirm previous studies in regard to fluoride retention.

179. Effect of Inorganic Ions on Utilization of Fluoride in Rats.—*Martin J. Wagner and Joseph C. Muhler, Indiana University, Bloomington.* Previous experiments have demonstrated numerical differences in the metabolism of fluoride obtained from a pure fluoride solution or from a natural fluoride-containing water supply. The present experiments were designed to evaluate the effect of calcium, magnesium, phosphorus, and iron present in natural waters in inhibiting the utilization of fluorine by experimental animals. The criteria of metabolic availability are skeletal and soft tissue fluoride retention, fluoride excretion in the urine, and fluoride present in feces. Weanling rats were furnished measured quantities of drinking water containing fluoride alone or in addition supplemented with calcium, magnesium, iron, or phosphorus at levels comparable to those found in communal water supplies. Fluoride was furnished at levels of 1 to 2 ppm fluoride as sodium fluoride. The results of these studies indicate some trend toward inhibition of fluoride utilization by the presence of these inorganic ions, as measured by total carcass fluoride retention. No significant effects on soft tissue fluoride retention have been noted.

180. Mechanism of Phosphate and Fluoride Exchange in Mineralized Tissues.—*Harold G. McCann and Elizabeth H. Fath, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.* The exchange of $P^{32}O_4$ ion and of fluoride ion with synthetic hydroxyapatite and powdered, defatted enamel, dentin, and bone was studied for

periods up to 140 days. With hydroxyapatite, enamel, and ashed bone, a considerable part of the total exchange takes place within the first 24 hours and is attributed to surface exchange. The exchange during the next 4 months is very gradual and is most probably a thermal diffusion process which has been termed recrystallization. The per cent phosphorus exchanged after 4 months was about 2.5 per cent with enamel and 4.0 per cent with this particular hydroxyapatite. The rate of exchange with dentin and bone was much more gradual than in the case of enamel or hydroxyapatite because of the much larger surface areas, although the amount of exchange was considerably greater after 140 days (dentin 25 per cent, bone 33 per cent). There was no rapid change in rate at any point and about a month was required before the rate was comparable to the very gradual exchange exhibited by hydroxyapatite and enamel after the first 24 hours. On the basis of available surface areas it is suggested that the diffusion of ions through the organic matrix of calcified tissues is the rate-determining factor in the exchange of $P^{32}O_4$ and fluoride ions with bone and dentin.

181. New Approach to Topical Application of Fluoride.—*Charles W. Gish, Charles L. Howell, Indiana State Board of Health, Indianapolis, and Joseph C. Muhler, Indiana University, Bloomington.* Each year for 3 years, approximately 550 second grade children, ages 7 to 8 years, have been divided into 2 groups following a clinical examination for dental caries. Both groups were balanced according to dental age and past caries experience at the time they entered the study. Each group of new second grade children received either a single application of an 8 per cent stannous fluoride solution or 4 applications of a 2 per cent sodium fluoride solution. The group receiving the stannous fluoride is treated once each year, while those receiving sodium fluoride are treated every third year. The data at the end of 3 years indicate that a single application of a freshly prepared 8 per cent solution of stannous fluoride applied once each year is significantly more effective in reducing dental caries in children than a series of 4 applications of a 2 per cent sodium fluoride solution applied every third year.

182. Effect of a Single Topical Fluoride Treatment in a Fluoride Area.—*Joseph C. Muhler, Indiana University, Bloomington.* Previous clinical studies have suggested that a single application of a concentrated solution (8 or 10 per cent) of stannous fluoride significantly reduced the dental caries experience of both children and adults. These studies were conducted in an area where the communal water supply was fluoride deficient, and it was of interest to determine if a similar effect would result if the children resided in an optimal fluoride area. Due to convenience, Indianapolis was chosen for the "fluoride city," even though this city has been fluoridating its water supply for a period of only 6 years. A total of 600 children, ages 5 through 12 years, were divided equally according to previous caries susceptibility into 2 groups. Group I received a single topical stannous fluoride application of an 8 per cent solution once every 6 months, and Group 2 received a single application of distilled water once every 6 months. Both treatments were given following a thorough dental prophylaxis. The results indicated a significant reduction in the dental caries experience both after 6 months and after 1 year in the group receiving the single application of stannous fluoride.

183. Topically Applied Potassium Fluorostannite. Three Year Results.— *Charles L. Howell, Charles W. Gish, Indiana State Board of Health, Indianapolis, and Joseph C. Muhler, Indiana University, Bloomington.* Approximately 900 children, ages 7 to 14 years, were divided into two groups following

a clinical examination for dental caries. Both groups were balanced according to dental age and past caries experience at the beginning of the program. Following a thorough prophylaxis, Group I received 4 applications of a 4 per cent potassium fluorostannite and Group II 4 applications of distilled water. The results at the end of 1 and 2 years have already been presented, and indicate a significant superiority of the potassium fluorostannite over the control in reducing the dental caries experience. After 3 years, the children were again reexamined. The data similarly indicate that the potassium fluorostannite significantly reduces the dental caries experience.

184. Effect of Previous Exposure to Fluorine and Age on Fluoride Metabolism.—*George K. Stookey, Martin J. Wagner, and Joseph C. Muhler, Indiana University, Bloomington.* In general, it has been concluded that fluoride storage parallels skeletal growth rate if the fluoride intake remains relatively constant. However, no studies have been reported in the rat which measure the effects of previous fluorine experience over long periods and the degree of skeletal maturity on skeletal retention and fluoride metabolism. Four groups of albino rats received 0.15 mg. fluoride according to the following schedule: (1) by stomach tube daily for 100 days; (2) during metabolism studies conducted during ages 30-36 days and again when at 130-136 days; (3) during the initial metabolism study period, but not during the final period; (4) during the final metabolism study period; and a fifth group which received no fluoride, serving as control. After the metabolism studies at the end of 100 days, the animals were sacrificed and analyzed for fluoride. The data demonstrate the decreased rate of storage by adult animals compared to the retention rate of rapidly growing young animals. In addition, the effect of previous fluoride experience is superimposed on the age effect and acts to oppose further storage of fluoride perhaps by mass law effects. As a result the rate of fluoride retention is least in adult rats with a history of fluoride ingestion.

185. Fluoride Retention and the Role of Ascorbic Acid in Fluorine Retention.—*Louis B. Spear, Jr., and Joseph C. Muhler, Indiana University, Indianapolis.* Previous studies have suggested that ascorbic acid increases the retention of fluoride in the skeleton, heart, and kidney of the guinea pig. Repeated studies, using a variety of different experimental variations are needed before this effect can be definitely stated, however. In order to learn more about this relationship, the metabolism of fluorine (fluorine excreted in urine, found in feces, and retained in skeleton) in guinea pigs, receiving varying levels of ascorbic acid and fluorine, was studied by methods previously described. The effect of fluoride and ascorbic acid on skeletal growth was also evaluated by determining the ash content of the femurs. The results suggest that ascorbic acid is related to fluorine retention in the guinea pig, since the retention of fluorine is greater where the ascorbic acid content of the diet is increased above the minimal required level.

XVIII. Oral Environment and Caries. March 22, Afternoon, Section B.

186. The Adhesion of Foodstuffs to Teeth.—*Robert C. Caldwell, University of Alabama, Birmingham.* A tensile strength measurement has been developed using the Scott I.P. 4 Tester, modified to measure the strength of food to tooth enamel bonds. Enamel surfaces are polished flat and the test food mixture is used to "glue" two enamel surfaces together. The force required to separate the teeth gives a measure of the stickiness of the food. Food mixtures of corn flakes, caramel, bread, etc., have been prepared in vitro by mixing with water or saliva. The same foods have been removed from tooth surfaces in vivo

after chewing and the different values for adhesion noted. Some representative values reveal that after chewing corn flakes, the material as it is swallowed has very little adhesion (> 1 lb./sq. in.) while the material removed from the teeth has considerable adhesion (6.8 ± 2.0 lb./sq. in. to 9.6 ± 2.9 lb./sq. in.). Saliva-corn flakes mixtures in vitro have less adhesion to enamel than water-corn flakes mixtures at the same dilutions. Caramel soaked in water for 2 minutes has slightly less adhesion than when soaked in saliva (9.8 ± 1.1 lb./sq. in. vs. 11.1 ± 0.7 lb./sq. in.). Caramel removed from the teeth in vivo had a value of 9.5 ± 0.9 lb./sq. in. The apparatus is being used to evaluate the effectiveness of organo-silicon, organophosphorus, and organotitanium treatments to enamel surfaces intended to reduce the adhesion of foods to teeth.

187. Attempts to Measure Total Acid Potential of Foodstuffs.— *D. J. Beck, D. I. Bayne, G. H. Davis, and B. G. Bibby, Eastman Dental Dispensary, Rochester, N. Y.* Work has been directed toward finding a more suitable technic for rapidly measuring acid production from common foodstuffs by saliva. A technic of neutralization with alkali at regular intervals during the course of fermentation was used, as it was felt this technic would give a more accurate measure of the total acid potential of foodstuffs. The technics of titration at the end of the test period only and measuring pH changes during fermentation were also employed. From the results achieved using these methods the following conclusions were drawn. Regular neutralization during the course of fermentation yields much higher titratable acidity than does terminal neutralization alone. The initial pH of the substrate and the substrate/saliva mixture greatly affects the fermentation, acid production being less from an acid substrate. Aeration during fermentation has no apparent effect on acid yield. Salivary sediment causes a more rapid initiation of fermentation than does whole saliva, but total acid production is greater from whole saliva. In an effort to explain these differences, buffering capacity and the effect of amylase were studied. These studies show that whole saliva and salivary supernatant have very similar buffer curves, but sediment has little buffering capacity. The addition of amylase to sediment does not apparently enhance fermentation.

188. The Effect of Peptides on Acid Formation in Sugar Saliva Mixtures.—*R. Q. Blackwell and L. S. Fosdick, Northwestern University Dental School, Chicago.* Pooled caries active saliva was divided into 10 ml. aliquots. To each tube was added 0.1 Gm. of sucrose and 0.1 Gm. of powdered human enamel. In each series, two of the samples were used as controls, while the other samples had known amounts of added test peptide. Each concentration was run in duplicate. The tubes then were incubated at 37.5° C. for 4 hr., centrifuged, and the clear supernatant analyzed for calcium. The difference between the calcium content of the fresh and the incubated samples is a measure of the acid formed during the incubation period. Peptides for the test were secured: (1) from this laboratory by synthesis and by partial protein hydrolysis; (2) from commercial sources, and (3) from numerous educational and industrial laboratories. Most of the peptides studied were without effect, but a number were found to be effective in concentrations as low as 25 mg./100 ml. saliva.

189. The Effect of Saliva on the Rate of Acid Formation in Dental Plaques.—*Harold R. Englander and Irving Shklair, Great Lakes, Ill., and Leonard S. Fosdick, Northwestern University Dental School, Chicago.* Dental plaques from certain locations of the upper molars were removed before and at 5-, 10-, 15-, and 20-minute intervals after the application of a 50 per cent sugar solution. The pH was determined on each sample after which it was analyzed

for lactate acid. The free lactic acid and the neutralized lactic acid of each sample was calculated by means of the Henderson-Hasselbalch equation $pH = pk_1 + \log \frac{BA}{HA}$. Two series of plaques were removed from each individual. The first series was performed under conditions which permitted free access of the saliva while, in the second series, no saliva was permitted to contact the areas under study. The pH and lactate content of the plaques which were taken in the presence of saliva were compared with those taken in the absence of saliva. It was found that in caries-active subjects the plaques remained acid for a longer period of time in the absence of saliva, although the total amount of lactate formed was approximately the same in the presence as in the absence of saliva. On the basis of the results, it is concluded that the saliva plays a very important role in the neutralization of acids in dental plaques. The results further indicated that, under normal circumstances, very little of the lactate formed is washed from the area by the saliva or metabolized by the organisms present.

190. Effect of Temperature on Powdered Enamel Solubility Reduction by Sodium N-Palmitoyl Sarcosinate.—*Joseph Tonzetich, Alexander W. Bouchal, William J. King, and Virgil J. Richter, Colgate-Palmolive Company, Jersey City.* Information was sought relative to (1) the temperature of the enamel surface "in vivo," (2) the influence of temperature on the reaction of sodium N-palmitoyl sarcosinate with enamel, and (3) the effect of temperature on acid decalcification of sarcosinate-treated enamel. The various "in vivo" tooth surfaces maintained near human physiologic temperature under almost all conditions studied. Both the reaction of sodium N-palmitoyl sarcosinate with enamel and acid decalcification of sarcosinate treated enamel were thermosensitive. Differences were noted in the effect of 0.1 per cent and 1.0 per cent concentrations of the sarcosinate. The 0.1 per cent sodium N-palmitoyl sarcosinate solutions at 25° to 50° C. conferred a high degree of protection to enamel against the solvent action of acetate buffer at 35° C. In contrast, the 1.0 per cent concentrations above 30° C. were relatively ineffective against acetate buffer. Temperature variations during the buffer treatment were found to be more critical than the temperature differences during enamel treatment with sodium N-palmitoyl sarcosinate. Both the 0.1 and 1.0 per cent sodium N-palmitoyl sarcosinate concentrations effectively reduced enamel dissolution in acetate buffer at temperatures above 30° C. and 32.5° C., respectively. At lower temperatures of decalcification the effectiveness of sodium N-palmitoyl sarcosinate was abruptly reduced. These data indicate the importance of considering the thermosensitive behavior of enamel-chemical reactions in assessing the possible anticariogenic capacity of the tested agent.

191. Influence of Film Thickness on Resistance to Glycolysis Inhibition.—*R. S. Manly, Tufts University School of Dental Medicine, Boston.* Sixty-two chemicals, selected because of initial promise as glycolysis inhibitors, were tested for their inhibitory action on 3 different thicknesses of thin films of salivary sediment. Film thicknesses of 0.2, 0.5, and 0.9 mm. were prepared in contact with a glass electrode and supported by stiffened nylon mesh. After the pH drop had been measured in a control solution, the coated electrodes were transferred for 50 minutes into a 1 per cent solution of the test chemical containing 20 per cent or less of propylene glycol as an accessory solvent. Next, the electrodes were transferred to the control solution for 1 hour to allow attainment of a new steady state. The percentage recovery was calculated from the ratio of the second to the first control pH differentials. The results were grouped

according to the recoveries at 0.2 mm. thicknesses and averaged in 5 groups. Recovery was generally proportional to thickness. The most inhibitory group of chemicals were affected to the greatest extent by change in thickness. The average recovery for 0.2 mm. films was 6 per cent, for 0.5 mm. films it was 49 per cent, and it became 78 per cent for 0.9 mm. films. The groups showing more than 40 per cent recovery on 0.2 mm. films possessed little or no inhibitory action when tested on 0.9 mm. films.

192. Dental-Caries Experience and the Oral Environment.—*G. H. Rovelstad and J. H. Geller, Dental Research Laboratory USNTC Bainbridge.* A group of young male adults (A), free from evidence of dental-caries experience at the time of entering the Receiving Center, have been under study since early 1956. A series of bacteriologic and biochemical tests have been conducted on the saliva of these young men for the purpose of determining oral characteristics. Two other groups were also selected and subjected to the same tests: one (B) a group of naval personnel of average dental conditions and the other (C) a group of naval recruits having rampant dental-caries experience prior to time of entrance. Three samples of saliva were taken from each subject and the following tests were conducted on each sample: lactobacillus count, Snyder test, desoxycholate agar plate count, Chapman's medium plate count, litmus milk test, nutrient broth turbidity test, optical density of nondialyzable saliva at 280 mμ wave length (protein), optical density of dialyzable saliva at 288 mμ wave length (uric acid), optical density of 1 N citrate solution of acetone precipitate of supernatant at 263 mμ wave length (nucleoprotein), optical density of saliva supernatant at 405 mμ wave length (blood), dissolved solids in supernatant sediment volume, and sediment decomposition. A significant relationship to the dental caries experience of the selected groups occurred in the Snyder test, lactobacillus test, litmus milk, desoxycholate agar count, optical density at 280 mμ dialyzable saliva, optical density at 405 mμ, and amount of sediment decomposition of saliva.

193. The Progression of Carious Lesions by Surfaces.—*J. A. MacLaughlin, F. R. Shiere, and H. R. Fogels, Tufts University School of Dental Medicine, Boston.* The purpose of this investigation was to study the caries incidence and dental care on the first permanent molar by surfaces in a specific geographical area. The examinations were made at yearly intervals on a total of 401 patients from 5½ to 10½ years of age. The children who were examined live in an industrial city in the state of Rhode Island. This program included full mouth x-rays and an oral examination. In order to evaluate individual surfaces, the IBM mark sense system was utilized. The amount of dental care received by the subjects is expressed by the number of fillings in contrast to the lack of dental care expressed by untreated carious lesions. A total of 277 sound first permanent molars were present at the first examination. At the second examination, the analysis showed on the occlusal surface a total of 125 DF surfaces, 93 decayed, 32 filled, expressed as a D/F ratio of 93/32. The ratio for mesial was 10/3, for distal 16/5, for buccal 82/20, and for lingual 79/6. Apparently surfaces other than occlusal receive less adequate care. In addition, an analysis was made of the relationship of the time delay between occlusal and mesial decay which suggests that, from a public health viewpoint, restoration of mesial surfaces should be recommended when occlusal restorations are made.

194. Sodium N-Lauroyl Sarcosinate Dentifrice; Effect on Dental Caries in Children.—*Lloyd A. Frasher, Riverside, Calif., and John W. Hein,*

Colgate-Palmolive Company in Cooperation with Rutgers University, New Brunswick, N. J. Three hundred and sixty-five children of Mexican extraction, attending 2 schools in Riverside, completed the 27-month experimental period. The control group of 184 children, averaging 8.35 years of age at the outset, were supplied a dentifrice containing as a detergent 2.0 per cent sodium salt of sulfated glyceride of coconut fatty acids. The 181 children in the experimental group, averaging 8.23 years of age at the outset, received the same dentifrice except for the substitution of 2.0 per cent sodium N-Lauroyl sarcosinate as the detergent. The drinking water of this area averages 0.9 ppm of natural fluoride. An initial, a 15-month, and a 27-month examination were conducted. The following statistically significant reductions in the incidence of dental caries over various periods of time were noted: in respect to DMF teeth, 0-15 months –53 per cent, 15-27 months –41 per cent, 0-27 months –48 per cent; in respect to DMF surfaces, 0-15 months –45 per cent, 15-27 months –43 per cent, 0-27 months –43 per cent. The mean DMFT and DMFS per child per year in the control group were 0.9 and 0.6, respectively.

195. Dietary Habits and the Dental-Caries Experience in 200 Children. —*Ralph E. McDonald, Indiana University, and Algeria C. Zita, University of the East.* Previous studies have suggested a positive relationship between the amount of readily fermentable carbohydrate and the dental-caries experience. The frequency of eating and the amount of carbohydrate consumed between meals have also been considered in previous studies but the results have been controversial and contradictory. From 7-day diet records of 200 children, the frequency of eating was determined, and the total sugar intake and the between-meal sugar was calculated as to its carbohydrate equivalent of teaspoons of sugar. The dental-caries experience of these children was determined from the clinical examination records and periapical and bite-wing radiographs and recorded as DMFS. The average total sugar intake of these children was 164 teaspoons while the average between-meal sugar was 55 teaspoons. A correlation between the total sugar consumed and the DMFS showed that the relationship was not statistically significant. The total between-meal sugar intake showed the highest correlation with the DMFS. The frequency of between-meal eating did not show a statistically significant effect on the dental-caries experience. A slight negative relationship was obtained between the total sugar intake and the milk intake, indicating that as the milk intake tends to increase, the sugar tends to decrease and vice versa. A multiple correlation between DMFS and the total sugar and total milk intake showed a negative correlation coefficient.

196. Dental Evaluation of 200 Public School Children in Salem, Massachusetts.—*M. Michael Cohen, Wm. A. Curby, and Richard M. Winer, Tufts University School of Dental Medicine, Boston.* In an investigation of 200 seventh grade pupils, ages 11-14, in the Salem, Massachusetts, Public Schools, the following data are compared: socioeconomic level of child; extent of previous dental care; oral hygiene; number of primary and permanent teeth present; decayed, missing, and filled teeth; calculus; materia alba; periodontal pockets; gingival recession; malocclusion; stain, location, and color; gingival inflammation, degree and location; and gingival enlargement, degree and location. In addition to high DMF counts, gingival inflammation and gingival enlargement were prevalent. A rather high percentage of the children showed no previous history of dental care. The methods employed in analysis of these children are shown to be simple, efficient, and rapid enough to make epidemiologic studies practical.

197. Preliminary Interrelational Study of Dental Caries in 1,800 Adult Patients.—*J. H. Manhold, Jr., Seton Hall College of Medicine and Dentistry, Jersey City.* Validity of the concept of bilateral symmetry of dental caries has long been controversial. Studies have demonstrated enough asymmetry to make conclusions, based on the hypothesis, somewhat suspect. The present study employs 1,800 cases (50,400 individual teeth) (1) to test the reliability of other, smaller population studies, and (2) to attempt to ascertain *which pairs* of teeth would be *most* reliable for use in studies requiring employment of this concept. The adult male patients' mouths were examined clinically and roentgenographically. Charts were made of their dental condition on a 28 tooth basis and, by assigning numerical surface caries ratings and employing IBM procedures, statistical analyses were conducted. Results verified (1) a high degree of correlation between bilateral pairs of teeth (C.R. = 6.36, sig. > 0.001 between bilateral and all other pairings), (2) no relationship between opposing pairs of teeth (C.R. = 1.60, sig. < 0.10 between opposing and all other pairings). Results further demonstrated (1) a superiority of lower and upper posteriors in regard to bilateral symmetry reliability (C.R.'s considerably below 1.00 as against 1 + in many superior pairs), and (2) some superiority of reliability of lower second molars and first and second premolars for use in bilateral symmetry of dental caries study (C.R.'s $\cong 0.30$ indicating no significant differences between bilateral pairs).

XIX. Cleft Palate and Histologic Studies. March 22, Afternoon, Section C.

198. Effects of Sialoadenectomy on Periodontal Tissues of the Hamster.—*Om P. Gupta and Harry Blechman, New York University, College of Dentistry, Murry and Leonie Guggenheim Foundation, New York.* Seventy-five, 35-day-old hamsters, weighing approximately 100 Gm., were divided into 6 comparable groups. The major salivary glands of the animals of 3 of the groups were removed while the remaining 3 groups served as the controls. Three different diets, No. 4 ground laboratory chow and laboratory chow pellets were given ad libitum to each desalivated group and its control. Animals were sacrificed after 12 weeks and the periodontal lesions evaluated according to the method of Gupta and Shaw. The desalivated animals in all the groups showed a higher incidence in the number and extent of periodontal lesions in both the soft and hard tissues than their controls. The increases in the number and extent of soft and calcified tissue lesions for the desalivated animals on diets No. 4 and laboratory chow were of moderate to high statistical significance. The difference in the number of soft tissue lesions for the animals on ground laboratory chow was of moderate statistical significance while it was of no significance for the extent of lesions. However, highly statistically significant differences were observed for the number and extent of calcified tissue lesions. Gross clinical findings were substantiated by histologic examination of the periodontal tissues.

199. Characteristics of a Bovine Submaxillary Gland Extract Used for Calculus Formation.—*Frank J. Draus and S. Wah Leung, University of Pittsburgh School of Dentistry, Pittsburgh, Pa.* Fresh bovine submaxillary glands were minced in a Waring blender at room temperature and extracted with distilled water for 24 hours at 4° C. The pH remained between 6.9 to 7.1. Following the extraction, the suspension was filtered with the aid of Celite 535 and the clear filtrate lyophilized. The average yield from the lyophilization was 4.5 per

cent, on a wet gland basis. The fluffy light brown material which was obtained became extremely tacky on wetting, and was soluble in water to 1 per cent. It gave positive Molisch and biuret tests. The viscosity of a 0.1 per cent solution of the extract was comparable to that of human saliva; and a 1 per cent solution gave a viscosity 5 times that of saliva. The lyophilized extract did not lose any of its viscosity character on standing at room temperature for 3 months. The extract on ashing yielded a residue of 13.75 per cent. Determination of Ca and total P on the ash indicated 5.6 per cent for the former and 25.2 per cent for the latter. The total nitrogen content was 10.20 per cent as determined by the Kjeldahl method. Paper chromatography showed the presence of N-acetylglucosamine, glucosamine, and glucuronic (galacturonic) acid, plus a protein component. The ultracentrifuge pattern on the extract indicated two components, with molecular weights below 500,000. The extract, fortified with Ca and P in a buffered system was employed as the substrate for the formation of synthetic calculus.

200. Calculus Formation From a Substrate Containing a Bovine Submaxillary Gland Extract.—*S. Wah Leung and Frank J. Draus, University of Pittsburgh School of Dentistry, Pittsburgh, Pa.* A bovine submaxillary gland extract fortified with Ca and P in a buffered solution was tested as the substrate for producing calculus-like deposits on human teeth, employing the dipping technic. In a typical experiment, a 0.1 per cent solution of the bovine gland extract was made up in a phosphate buffer pH 7.5 and adjusted to contain 5 mg. per cent Ca and 15 mg. per cent P. A final pH of 6.8 was obtained by bubbling in CO_2 This solution was then placed in the dipping apparatus, at a constant temperature of 37° C., and the dipping rate regulated to allow the teeth to be in contact with the solution 30 seconds for each cycle of 1 minute. A control solution without the extract, but otherwise identical to the test solution, was run concurrently on another set of teeth. The solutions were changed daily, the final pH being around 7.5 after 24 hours. The testing continued for 4 weeks, after which time the deposits which had formed on the teeth were analyzed. The deposit obtained from the control teeth without the extract had an average dry weight of 8.51 mg. per tooth while the deposit from extract-treated teeth averaged 12.15 mg. per tooth. Analysis showed 30.5 per cent Ca, 17.8 per cent P, and Ca/P ratio of 1.71 for both the experimental and control deposits. The organic content, as determined by ethylenediamine extraction, was 22.3 per cent for the material from the extract-treated teeth, and less than 1.0 per cent for the control deposit. X-ray diffraction patterns showed the inorganic fraction of the materials to be similar to hydroxyapatite.

201. Formation and Stability of Whitlockite and Octocalcium Phosphate, Two Components of Salivary Calculi.—*Otto R. Trautz and Emma Fessenden, College of Dentistry, New York University, New York.* A report on x-ray diffraction and chemical experiments. Whitlockite is the hexagonal β-$Ca_9(PO_4)_6$. Up to one sixth of the Ca atoms can be substituted by Mg at 900° C. Three new phases have been discovered between whitlockite and $Mg_9(PO_4)_6$. Magnesium substitution (16 atomic per cent) reduces the a and c axes (1.0 and 0.42 per cent). From the shrinkage the Mg concentration can be determined. Up to 3.5 weight per cent Mg has been found in the whitlockite of dental calculus. Pure Ca whitlockite is hydrolyzed by water; but whitlockite with 4 weight per cent Mg is not decomposed when suspended in boiling water for several months or extracted in a Soxhlet apparatus. Whitlockite with 1 per cent Mg is hydrolyzed to some extent. The experiments demonstrate the increased stability of magnesian whitlockite and explain the possibility of its

formation from oral fluids and the frequency of its occurrence in the calculi. Octocalcium phosphate, $Ca_8H_2(PO_4)_6 \cdot 6H_2O$, could best be precipitated between pH 5.6 and 6.0. Below this range it is contaminated with more brushite, above pH 6 it is faster hydrolyzed to apatite. Elevation of the temperature to 40° C. impedes the crystallization of brushite. Thus, mouth conditions seem quite favorable for the formation of the octocalcium phosphate. It is quite stable in air. After 60 hr. at 110° C., it has lost only half of its water content. Further heating at 605° C. for 16 hr. removed the remaining molecules of crystal water. Electron micrographs picture the precipitate as very thin films.

202. A Possible Factor in Cleft Palate Etiology.—*James K. Avery, James D. Happle, and William C. French, School of Dentistry, University of Michigan, Ann Arbor.* The nasal region of 5 normal appearing and 4 cleft lip and palate human embryos, ranging in size from 19 to 46 mm., crown-rump length, was studied. Their ages represent the seventh through tenth prenatal weeks when the nasal cartilages and subadjacent palate form. The nasal capsular cartilages represent the maxillary facial skeleton during the prenatal period of palatal formation. Microscopic study of the tissue sections and reconstruction of the nasal capsules by the wax plate method revealed considerable variations in structure between the normal and the cleft lip-palate series. The nasal capsule in the seventh week cleft lip-palate specimen had not differentiated. This is considerably behind normal development at this age. The 2 bilateral cleft lip and palate specimens had nasal capsules which were approximately one half to two thirds that of the normal average length, whereas the capsule of the unilateral cleft palate embryo was longer and within normal size range. Defects were apparent along the inferior and anterior parts of the septal and lateral nasal cartilages. The nasal septum was bifurcated in the midsagittal plane near its anterior limits so that each lateral nasal cartilage appeared attached to an individual half of the septum. Since the nasal capsular cartilages represent the embryonic facial skeleton and differentiate prior to palatal formation and fusion, it is likely that this defective cartilage development may be a causative factor in abnormal palatal development.

203. Intelligence Quotient of Children With Congenital Cleft Palate.—*Lewis J. Rattner, William J. Carter, University of Kansas City School of Dentistry, and Loraine Pilkey, Children's Mercy Hospital, Kansas City, Mo.*— Review of the literature of the I. Q. of children with congenital cleft palate revealed sparse investigation and diverse results. Intelligence quotient values were obtained on 17 randomly chosen patients from the Children's Mercy Hospital Dental Clinic. There were 12 males and 5 females ranging in age from 6 to 15. The test used was the Wechsler Intelligence Scale for children which separated the verbal and performance area levels. The results are given in table.

	MEAN	STANDARD DEVIATION
Verbal scale	98.00	11.23
Performance scale	99.94	15.56
Full scale	98.70	12.50

Statistical evaluation of results revealed no significant difference between the I. Q. of verbal and performance areas for cleft palate vs. normal children. Mann-Whitney U test was used to determine significance between I. Q. values for 6 Class III and 9 Class IV cleft palate children. No significant correlation was found.

204. Condylar Osteoclast Counts on Vitamin C Deficient Guinea Pigs.—*Ben W. Warner, Hugh I. Myers, and Viola D. Flanagan, University of Kansas City, School of Dentistry, Kansas City, Mo.* Four-week-old guinea pigs were fed a standard scorbutic diet and green vegetables for one week. For the next 3 weeks, they were placed on the scorbutic diet alone and then sacrificed. The entire condyles were removed, fixed in formalin, decalcified electrolytically, embedded in paraffin, and serially sectioned in the frontal plane at 10 microns. Every tenth section was mounted and stained with hematoxylin-eosin. The fields (×440) were examined progressively from lateral to mesial borders and all osteoclasts noted in the head, neck, and beginning ramus. Results from 11 animals show that the counts in the experimental group are significantly lower than those made on control animals. Average of the total number of osteoclasts counted per condyle for the experimental group was 149 ± 37.7. In the same age control animals, these counts were approximately 4 times as great. There appeared to be a complete cessation of bone formation at 8 weeks. In the marrow spaces, many spicules of bone appear surrounded by accumulations of osteoblasts and osteoclasts. Osteoclasts cell shrinkage and pyknotic nuclei are accelerated in the experimental group. The greatest number of osteoclasts appeared in the zone of erosion among the spicules in the internal condylar neck region; less occurred at the lateral portions of the head of the condyle just underneath the periosteum, and the junction of the cartilage with the bone. This is a direct reversal of the process seen in normal animals.

205. Controlled Mechanical Stresses on the Maxillary Suture of the Rat.—*Charles J. Burstone and William Shafer, Indiana University, Indianapolis.* To study the effects of force variation and systemic factors on sutural expansion, a method was devised to deliver relatively constant forces to the maxillary suture of the rat. "Intermittant continuous" forces were produced by uniform rubber wedges maintained between the maxillary incisors. Maintenance of the wedge was further facilitated by the presence of mesial-distal holes through which a 0.016 stainless steel wire pierced tooth and rubber. This method was applied to animal groups comprising male albino rats of 2 months of age. It can be reported that relatively uniform results and durability of the appliance were found. Histologic changes in the maxillary suture with low order forces were: (1) increased cellularity, (2) the formation of thin layers of osteoid on bone surfaces proximal to the suture, (3) moderate increase in sutural width, and (4) orientation of collagen fibers parallel to the direction of the force. Higher order forces were characterized by greater increases in cellularity, osteoid, sutural width, and fiber orientation with the increased osteoid formation exhibiting itself as osteophytes. This technic offers an approach for the study of systemic factors on bone dynamics. At the present, the method has been applied to a consideration of thyroid function on sutural expansion.

206. Blood Flow in the Mandibular Artery of Anesthetized Dogs.—*Jack G. Bishop, J. Lester Matthews, Homer L. Dorman, and E. Earl Moore, Baylor University College of Dentistry, Dallas.* The blood flow through the right mandibular artery has been measured on 6 large healthy dogs. These measurements were accomplished by use of a simplified electromagnetic blood-flow meter which necessitates cannulation of the vessel. Simultaneous recording of blood pressure by means of a Statham strain gauge and amplifier were made in order to permit calculation of peripheral resistance. Control levels showed an average of 8 c.c./min. blood flow with a pressure of 85 mm. Hg and yielded a PRU of 12. Injections of epinephrine increased PRU to 70. Acetylcholine

injections, on the other hand, reduced the PRU value to 3.3 within 1 minute. Radiographic analysis during infusion of Thorotrast revealed that the tissues supplied by this artery are largely unilateral and do not include the tongue. The establishment of the normal blood flow in this vessel should provide a basis for better evaluation of the vascular involvements associated with dental pathology.

207. A HISTOPATHOLOGIC EVALUATION OF NERVE SCLEROSANTS.—*Allen J. Koslin, State University of Iowa, Iowa City.* In recent years, some clinicians have used sodium psylliate as a neural sclerosant, in lieu of absolute alcohol, on an empirical basis. The relative efficacy of these and certain other sclerosing agents warrant experimental study and evaluation. This was an animal experiment using rabbits to determine by histopathologic study the effects of various agents on a mixed nerve and surrounding tissues. The sciatic nerves were exposed and a fixed quantity of solution deposited on them. All incisions were closed. The agents used were absolute alcohol, sodium psylliate, calcium penicillin, calcium hydroxide, and sodium dicetyl phosphate in olive oil. A series of N/10 saline controls were included. Two series of rabbits were used for each agent and representative biopsies were performed at 1, 2, 3, 4, 6, 8, and 12 week intervals. Microscopic sections, stained with hematoxylin-eosin or luxol fast blue myelin stain, were prepared and studied. N/10 saline produced no evidence of tissue damage or inflammatory response. Saturated calcium hydroxide solution caused a focal coagulation necrosis of the nerves and severe associated tissue damage. Absolute alcohol was more effective than sodium psylliate as a neural sclerosant and resulted in slightly less surrounding tissue damage. Sodium dicetyl phosphate and calcium penicillin elicited marked foreign body reactions in the adjacent tissues without nerve damage. This study indicated that the most effective agent, of those tested, for clinical nerve blocks was absolute alcohol.

208. INVESTIGATION OF LOCALIZED ACTION OF SOMATOTROPIN ON THE TEMPOROMANDIBULAR JOINT.—*Richard Mackenzie and James K. Avery, School of Dentistry, University of Michigan, Ann Arbor.* A group of 19-day-old male and female hamsters was divided into 5 groups and administered 30 daily injections into the right temporomandibular joint. Animals of group A were injected with 20 mcg. of somatotropin in saline, adjusted to pH 8.1 with NaOH. The animals of group B were injected with 2 mcg. and the animals in group C, 0.2 mcg. of somatotropin. The animals in group D served as controls and were injected with a blank solution into the right temporomandibular joint. The blank solution was saline with the pH adjusted to 8.1. Group E animals were not injected and also served as controls for the study of the normal temporomandibular joint. All animals were weighed daily and at the end of the 30-day period were sacrificed. At the time of sacrifice the heart, liver, kidney, and spleen were examined and weighed. The dimensions of the right femur of each animal were taken and the heads were bisected and then radiographed. The areas of the mandibular rami and the temporomandibular joints were then decalcified, sectioned, and stained for histologic study. The following findings were observed. The hormone-injected animals gained more weight during the period of injection than the noninjected animals and their femurs were longer than the normal. The histologic sections of the temporomandibular joints revealed that chondrogenic activity was greater in the hormone-injected animals than in the normal with no visible difference between the injected and the noninjected joints. Radiographs revealed that the right and left mandibles of the animals receiving hormone were longer than the mandibles of both control groups; the

mandibles on the injected sides of the saline-control groups were shorter than those on the opposite sides. The results indicate that beef somatotropin has an increased growth effect on the Syrian hamster but that this effect is not more pronounced in the target area than elsewhere.

209. Progress of Eruption of the First Permanent Molars.—*H. R. Fogels and F. R. Shiere, Tufts University School of Dental Medicine, Boston.* This study is concerned with the first permanent molar, its emergence, complete eruption, and subsequent extraction from caries in a specific geographical location. The patients are residents of an industrial community in Rhode Island. They are of various ethnic backgrounds, mostly European. There were a total of 341 subjects, ranging in age from 5½ to 10½ years at the first examination. This is a report after 4 annual examinations. The examinations included full-mouth x-rays, clinical oral examination, and impressions for study models. In order to evaluate the progress of eruption, five categories were determined: (1) untreated, (2) cuspal emergence to one third of the crown, (3) more than one third to less than total emergence of the crown, (4) total emergence, and (5) extraction. The analysis of the data was completed by IBM machines. All fully erupted teeth at the first examination were excluded as were those which were extracted prior to that time. The examinations were made in the same month a year apart and the data were evaluated according to the birth year and month. The average emergence age for first molars was 6 years and 9 months for uppers and 6 years and 7 months for lowers. Full eruption was not completed until 7 years and 4 months for upper and 8 years for lower. By 10 years and 3 months, 8.0 per cent of lower molars were lost and by 10 years and 6 months, 3.2 per cent of upper molars.

XX. Titles Presented*

T210. Development of Teeth in Swiss Albino Mice. *Dimitri Afonsky,* Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.

T211. Caries Susceptibility in the Mouse. *Dimitri Afonsky,* Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.

T212. Effect of Desalivation in Reproduction. *Dimitri Afonsky,* Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.

T213. Citrate of Calcified Tissue. *W. D. Armstrong and Leon Singer,* University of Minnesota, Minneapolis.

T214. Fluoride Content of Sound and Carious Korean and Minnesota Teeth. *Leon Singer and W. D. Armstrong,* University of Minnesota, Minneapolis, and *Myung Jim Park,* Seoul National University, Seoul, Korea.

T215. Addition of Bacteriostatic Agents to Acrylic Resins. *Arthur N. Bahn,* College of Medicine, *Roger C. Michalsen and Maury Massler,* College of Dentistry, University of Illinois, Chicago.

T216. Effect of $CaHPO_4$ and NaF Dietary Supplements of Experimental Caries. *Peter Barnard and Erling Johansen,* University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

*These papers are not presented in person, although where vacancies may occur in the program due to extentuating circumstances, one or more of these may be read by the principal author

T217. Lymphoepithelial Lesions of Salivary Glands. *Joseph L. Bernier and Surindar N. Bhaskar,* Armed Forces Institute of Pathology, Washington, D. C.

T218. Phosphatase Activity of Ligated Submaxillary Glands of the Rat. *Theodore E. Bolden,* Seton Hall College of Medicine and Dentistry, Jersey City, and *J. P. Weinmann,* University of Illinois, Chicago.

T219. Incidence of Fractures of the Cementum in Human Dentitions. *Theodore E. Bolden,* Seton Hall College of Medicine and Dentistry, Jersey City, and *Joseph P. Weinmann,* University of Illinois, Chicago.

T220. Effect of Flavored Beverages, Orange Juice, Sugar Solutions on Rat Caries. *Michael Buonocore,* Eastman Dental Dispensary, and *Erling Johansen,* The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

T221. The Comparative Efficacy of Gum, Toothpaste, and Mouthwash. *Howard H. Chauncey,* Tufts University School of Dental Medicine, Boston.

T222. National Dental Evaluation of Children With Cerebral Palsy. *M. Michael Cohen, William A. Curby, and Manuel M. Album,* Tufts University School of Medicine, and School of Dental Medicine, Boston.

T223. Acid Formation in Saliva and Plaques. *H. Eggers-Lura,* Laboratory of the County Hospital, Holback, Denmark.

T224. Problems of Experimental Polycythemia. *Clark Eid and Robert J. Gorlin,* School of Dentistry, University of Minnesota, Minneapolis.

T225. In Vitro Reactions of Epithelium to Growth Hormone Injected in Vivo. *R. Gerstner, S. S. Stahl, O. Ioly, and E. O. Butcher,* The Murry and Leonie Guggenheim Foundation, Institute for Dental Research, and New York University College of Dentistry, New York.

T226. The Willmar Story: A Pilot Study in Mass Free Oral Cancer Examination. *Robert J. Gorlin and Lee Berglund,* School of Dentistry, University of Minnesota, and Minnesota Division, American Cancer Society.

T227. The Trace Element Selenium and Its Influence on Dental Caries Susceptibility. *D. M. Hadjimarkos,* University of Oregon Dental School, Portland, and *Carl W. Bonhorst,* University of Portland, Portland.

T228. Inhibition of Salivary Respiration and Fermentation by a Fluoridated Cationic Detergent. *Ulrich Hermann and Hans R. Muhlemann,* Dental Institute, University of Zurich, Switzerland.

T229. A Technic for the Filling of Root Canals in Hamster Molar Teeth. *Robert B. Hoek, Emmett R. Costich, and James K. Avery,* University of Michigan, Ann Arbor.

T230. Effect of Vitamin E Deficiency on the Enamel Organ. *J. T. Irving,* University of the Witwaterarand, Johannesburg, South Africa.

T231. Early Pulpal Calcifications of Permanent Teeth of Young Individuals. *Verda Elizabeth James,* University of Illinois, Chicago.

T232. Histogenesis of the Keratinized Enamel Cuticle. *Percy L. Johnson and G. Bevelander,* Murry and Leonie Guggenheim Foundation, Institute for Dental Research, College of Dentistry, New York University, New York.

T233. The Effect of Sodium Lauroyl Sarcosinate and Sodium Fluoride on Dental Caries in Rats. *Paul H. Keyes and Carl L. White,* National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.

T234. Double Mating of Hamsters to Elucidate Genetic Factors Influencing Dental Caries Susceptibility. *Paul H. Keyes and Johannes L. Scheltema,* National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.

T235. Diagnostic and Scoring Problems Related to Experimental Rat Caries. *Paul H. Keyes,* National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Bethesda.

T236. The Influence of Altered Physical State of the Diet Upon Dental Caries. *C. E. Klapper, and J. F. Volker,* University of Alabama, School of Dentistry, Birmingham.

T237. A Study of Ethylenediamine Treated Enamel. *Donald E. Knapp, James K. Avery, and Emmett R. Costich,* School of Dentistry, University of Michigan, Ann Arbor.

T238. Experimental Caries Inhibition and Simultaneous Enamel Solubility Reduction. *Klaus G. Koenig and Hans R. Muhlemann,* Dental Institute, University of Zurich, Switzerland.

T239. In Vitro Electrical Resistance Measurements of Teeth With and Without Silicone Film. *M. Krakowski, B. S. Savara, N. M. Phatak,* University of Oregon Dental School, and *J. Dement,* Dement Research Laboratories, Portland.

T240. Effect of Ultrasonic Cavity Preparation on the Periodontium of Dogs. *Harry Lyons, John J. Salley, and George W. Burke, Jr.,* Medical College of Virginia, Richmond.

T241. Endamoeba Gingivalis and Its Relation to Gingival Pathology. *Maurice A. Mazzarella and Noshi Monsour,* Naval Medical Research Unit No. 3, Cairo, Egypt.

T242. The Local Effect of Insulin on Wound Healing in Rats. *Govind B. Shankwalker,* Indiana University School of Dentistry, Indianapolis.

T243. Fluoride Tolerance: Action of Fluoride on Weight, Calcium Metabolism, and Calcification of the Bones and Teeth. *J. C. Muracciole,* Hospital Nacional de Odontologia, Buenos Aires, Argentina.

T244. Changes of Keratins Under Load. *H. H. Neumann,* Columbia University, New York, N. Y.

T245. A Quantitative Method for Screening Nutritional Symbionts. *Thomas A. Nevin,* National Institute of Dental Research, *T. Shiota and Edward G. Hampp,* American Dental Association at National Institute of Dental Research, National Institutes of Health, Bethesda.

T246. Hamster Gingival Plaque and Dodecyl Benzene Sulfonate. *Paul B. Risk and David F. Mitchell,* Indiana University School of Dentistry, Indianapolis.

T247. Further Studies of the Effect of Tobacco on Oral Tissues. *John J. Salley,* Medical College of Virginia, Richmond, and *Seymour J. Kreshover,* National Institute of Dental Research, Bethesda.

T248. Effect of Thyroxine and Triiodothyroacetic Acid on Incisor Eruption Rate. *Sheldon Schumer and Herbert Wells,* School of Dental Medicine, Harvard University, Boston.

T249. Effects of Burs, Airbrasive and Ultrasonic Devices on the Pulp of Rat Incisors. *Michel Serfaty, Maury Massler, and Verda E. James,* University of Illinois, Chicago.

T250. Response to Despeciated Calf Bone and Calcium Hydroxide Implants. *Govind B. Shankwalker and David F. Mitchell,* Indiana University School of Dentistry, Indianapolis.

T251. Sodium and Potassium Levels of Saliva and Salivary Glands Following Prolonged Stimulation. *Ira L. Shannon,* School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.

T252. Salivary Sodium, Potassium and Chloride Levels in Subjects Classified as to Caries Experience. *Ira L. Shannon,* Research Dentistry Division, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.

T253. The Temperature of Teeth and Gingiva. *Max Shapiro,* California Institute of Dental Research and Cedars of Lebanon Hospital, and *Ben H. Ershoff,* Western Biological Laboratories, Culver City, Calif., and Southern California School of Medicine.

T254. The Relationship of Non-Axial Forces in Deglutition to Periodontal Disease. *Irving M. Sheppard,* Dental Department, Montefiore Hospital, New York.

T255. Reaction of Orange-G With Bone and Dentin Collagen. *C. C. Solomons,* Joint Dental Research Unit of the Council for Scientific and Industrial Research and the University of the Witwaterarand, Johannesburg, South Africa.

T256. Organic Content of Enamel From Greek and British Teeth. *M. V. Stack and J. Halazonetis,* Dental School, University of Bristol, England.

T257. The Effects of Somatrophic Hormone on the Periodontal Structures of Normal Adult Male Rats. *S. S. Stahl,* Murry and Leonie Guggenheim Foundation, Institute for Dental Research, New York University College of Dentistry, New York.

T258. Effects of Protein Deprivation on the Periodontium of Young Adult Male Hamsters. *S. S. Stahl, S. C. Miller and E. D. Goldsmith,* Murry and Leonie Guggenheim Foundation, Institute for Dental Research, New York University College of Dentistry, New York.

T259. Pulpal Damage Under Extracoronal and Intracoronal Preparations. *Stanley D. Tylman, John M. Spence, Marvin B. Weiss, and Maury Massler,* University of Illinois, Chicago.

T260. Incidence of Parastic Protozoa in the Human Mouth. *Wayne W. Wantland, John W. Remo,* Illinois Wesleyan University, Bloomington, Ill., and *D. L. Winquist,* Dental Clinic, Bloomington.

T261. Color Reaction Between Auric Chloride and Diiodotryosine. *Joel Wisotzky and Joan D. Bell,* Division of Dental Medicine of the Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.

T262. Removal of Radioactive Fat and Protein From the Mouth. *W. W. Wainright,* Los Angeles, *F. K. Bauer and P. B. Thomas,* University of Southern California School of Medicine, Los Angeles.

T263. Effect of Fluoride on the Developing Teeth of Rats. *B. R. Bhussry,* University of Rochester, Rochester, N. Y.

T264. Ascending Pulpitis in Primary Teeth. *Viktors Kalnins,* School of Dentistry, Western Reserve University, Cleveland.

XXI Abstracts of Papers to Be Presented to the Dental Materials Group*

M1. Analysis of Methyl Methacrylate Copolymers by Vapor Phase Chromatography.—*John Strassburger and Gerhard M. Brauer, National Bureau of Standards, Washington, D. C., and Alphonse F. Forziati, American Dental Association Research Division, National Bureau of Standards, Washington, D. C.* Vapor phase chromatographic procedures have been used to identify and estimate quantitatively copolymeric components in acrylic resins. Copolymers containing methyl or ethyl methacrylate as major constituents were prepared by bulk polymerization. Copolymeric constituents included methyl acrylate, ethyl acrylate, acrylic acid, methacrylic acid, and some cross-linking agents. The polymers were pyrolyzed at 350° C. and the liquid pyrolysate was analyzed by vapor phase chromatography. Helium was used to carry the vapors through a column packed with dinonyl phthalate coated firebrick. Some of the pyrolysis products could be identified, aiding in the elucidation of the mechanism of the depolymerization reaction. The presence of copolymeric constituents was detected by comparing the chromatograms of the pyrolysis products from copolymers with those obtained from polymers prepared from a single monomer. From the characteristic times of appearance of additional peaks the monomers used in the copolymerization were identified. The presence of copolymers in poly (methyl methacrylate) which had been prepared by polymerizing methyl methacrylate monomer in the presence of 0.2 per cent or 1 per cent of various comonomers could always be detected. Quantitative determinations of copolymeric components such as methyl acrylate, ethyl methacrylate, or ethylene glycol dimethacrylate in methyl methacrylate resins have been accomplished.

M2. The Effect of Varying Peroxide and Polymer on the Rate and Degree of Polymerization of Polymethyl Methacrylate Slurries.—*John A. Cornell and Catherine M. Powers, H. D. Justi & Son, Inc., Philadelphia.* A number of new promoter systems have been suggested for use in dental plastics in the last several years. Many of the potential faults in these systems can be investigated by studying variations in a well-studied model system; benzoyl peroxide and dimethyl paratoluidine. Completeness of polymerization was measured by using a Rockwell M test at various times. Comparative results using different peroxides and percentages of peroxide are shown. Generally with one peroxide, completion of polymerization is proportional to rate of

*This meeting will coincide with that of the International Association for Dental Research. Due to late changes, the papers may not be presented in the same order as they appear here.

polymerization and also concentration of peroxide. However, among different peroxides, completeness of polymerization is not necessarily proportional to rate. Some theory of rate vs. completion is discussed.

M3. Physical Properties of Denture Base Reliners.—*Gerhard M. Brauer and Eli White, Jr., National Bureau of Standards, Washington, D. C.* Physical properties of 6 commercial hard setting reliners have been studied. Since these materials are plasticized room temperature curing acrylic resins the tests described in A.D.A. Specification No. 12 were used whenever applicable. All materials pass the packing test and their initial consistency is adequate. Curing time was most conveniently determined by measuring the time at which the peak temperature occurred. Peak temperatures vary from 30° to 53° C. for resins cured at 23 ± 2° C. and from 59° to 79° C. for those polymerized at 37 ± 1° C. Results of a test designed to determine tackiness, flow, and detail indicate that some materials still show considerable flow at the time suggested in the manufacturer's instructions for the removal of the relined denture from the mouth. Details of the final impression made by the reliner are sometimes poor. Some of the reliners possess poor color stability. The reliners comply with the A.D.A. Specification No. 12 requirement for sorption and most of their solubilities are within the specification limits. Sorption and solubility depend somewhat on the pressure applied on curing but are affected only slightly by surface porosity of the polymer. The effect on the transverse strength of acrylic resin relined with these materials was studied. Plasticizers in the monomeric and polymeric components of the reliners were determined quantitatively after identification by infrared absorption.

M4. Physical Properties of Denture Base Materials.—*John L. Tucker, John A. Cornell and Catherine M. Powers, H. D. Justi & Son, Inc., Philadelphia.* Impact testing by using a falling ball indicates that crosslinked polymethyl methacrylate polymer resists breakage of repeated blows better than an equivalent uncrosslinked polymer. The impact resistance depends upon the degree of crosslinking. Plasticizer also has an effect on impact resistance. Some results with different molding conditions are discussed. Effect of some fillers is shown, and the effect of fillers on toughness properties is discussed.

M5. An Automatic Shot Loading Machine for Transverse Tests of Denture Base Resins.—*W. D. Kimmel, The L. D. Caulk Company, Milford, Del.* When shot is loaded by hand in making the transverse deflection test of the A.D.A. Specification No. 12, it is difficult to obtain an even flow of shot. A mechanical method has been devised to load the specimen. This consists of a small screw conveyer driven by an adjustable speed motor which pulls shot from a reservoir at a constant rate period. The speed of the conveyer is adjusted to give 500 plus or minus 3 Gm. of shot in 25 seconds. A timing switch is set to supply current to the motor driving the conveyer for 25 seconds in each minute. This allows the operator time to read and record the deflection just before the loading is begun each minute and assures a constant rate of loading. Data are given on the accuracy of the loading device.

M6. Composition of Denture Reliners Used by the Public.—*Harold J. Caul and John W. Stanford, American Dental Association Research Division, National Bureau of Standards, Washington, D. C.* Denture reliners used by the public are very different from those used by the dental profession for relining or rebasing artificial dentures. The constituents of these materials can be identified by infrared absorption procedures or by analytical organic methods.

In most instances, the reliners were placed in a still and gradually heated to temperatures as high as 350° C. At or below this temperature the constituents distilled or degradated and the individual components could be identified. Infrared proved to be extremely useful in identifying the constituents, especially the more complex ones. In most cases these reliners consist of a polymer such as methyl methacrylate, butyl methacrylate, vinyl acetate or cellulose nitrate and a plasticizer or solvent. Several contained natural gums instead of resins. Plasticizers such as glyceryl triacetate, dibutylphthalate, butyl phthalylbutylglycolate were found. Paraffin wax, cotton cloth, acetone and other solvents are also used in the manufacture of some of the reliners. It was possible in most instances to separate the major constituents of these reliners by extraction methods so that a quantitative estimate could be made. The chemical compositions of the reliners appear to be correlated with the affects of the reliners on the physical properties of denture base resins. Those containing solvents or plasticizers affect the physical properties of the denture base resin by lowering stiffness and strength and also cause warpage and crazing while the others do not.

M7. The Contour Meter.—*Gunnar Ryge and Carl W. Fairhurst, Marquette University, Milwaukee.* The "contour meter" is an apparatus for comparison of mucosal surface contour of impressions, models, and dentures. It is constructed to facilitate evaluation of dimensional behavior of the various materials employed in denture construction. Impressions, models, and dentures are positioned by means of three reference points which are transferred from impression to model to denture. The reference points makes it possible to orient the object to be recorded in a reproducible position in the contour meter, where measurements are recorded in a three dimensional coordinate system by means of micrometers. The reproducibility and accuracy of recordings were analyzed on the basis of a series of 3 recordings of a stone model including a total of 168 readings. The average deviation of experimental difference in positioning the models and recording the 56 points 3 times was 0.06 mm.

M8. Evaluation of Denture Adaptation.—*Carl W. Fairhurst and Gunnar Ryge, Marquette University, Milwaukee.* The mucosal surface of 27 stone models and the denture blanks processed thereon have been recorded by use of the contour meter. The denture blanks were recorded immediately after processing and after 10 and 30 days' storage in water at 37° C. Each recording included 59 corresponding points on each of the models and denture blanks, and denture adaptation was evaluated in terms of the variation between corresponding points on denture blanks and models. Denture blanks were fabricated with: (1) methyl methacrylate gel using an injection technic and wet and dry heat cure; (2) methyl methacrylate powder and liquid using a compression molding technic and wet and dry heat cure; and (3) styrene resin using an injection technic with preheated resin. It was found that denture adaptation as recorded immediately after processing was best for denture blanks processed by method 3. After 10 days' storage best adaptation was found using method 2, dry heat. After 30 days' storage, denture blanks processed by method 3 showed best adaptation. Dry curing of gel material resulted in slightly better adaptation after 30 days' storage than did wet curing of the same materials. Adaptation after 10 days' storage was superior to the adaptation after 30 days' storage, except for denture blanks processed by method 3.

M9. An Investigation of Corrosion of Certain Metallic Dental Materials.—*Marjorie L. Swartz, Ralph W. Phillips, and Mohamed Daoud El Tannir,*

Indiana University School of Dentistry, Indianapolis. An in vitro study was made to investigate the corrosion of various metallic alloys and of certain factors thought to influence it. As a basis for this study, the chemical nature of the corrosion produced in vivo was first determined through x-ray diffraction patterns made on the surfaces of amalgams and gold inlays taken from extracted teeth. The actual materials employed in the study were golds of high and low carat, zinc and nonzinc amalgam alloys, and a gallium alloy. Specimens of these materials were subjected to different concentrations of solutions of sodium sulfide, sodium chloride, and hydrogen peroxide. Distilled water and an artificial saliva were also utilized. The effects of exposure of the media to air and the role of galvanic action were also investigated. Such manipulative variables as residual mercury content, moisture contamination, polish, etc., as related to corrosion of amalgam alloys, were studied. X-ray diffraction patterns were obtained on some of these artificially corroded specimens. Analysis of in vivo corrosion and the data obtained from the in vitro tests indicate sulfides to be the most common source of corrosion except with the gallium alloy. It also appears that corrosion of metallic alloys is influenced by galvanic currents, pH of the surrounding environment, and exposure to air. In particular, all conditions which contributed to surface roughness decreased the resistance to corrosion.

M10. The Response of Restorative Materials to Thermal Variations.—*Frank H. Nealon, Cleveland.* A method of obtaining relative thermal absorption values for dental filling materials is described. The time that is required for the materials to show a temperature rise of varying amounts is recorded in table and graph form. Materials are in the order of their thermal absorption values. Thermal absorption is a prerequisite to thermal expansion. A comparison is made of materials with a high coefficient of thermal expansion and low thermal absorption to materials of reverse characteristics. The observations are significant because reflex action and normal physiology of the oral structures demand that temperature variations of the teeth and contiguous structures are transient and of short duration.

M11. Standardization of the Colors of Silicate Cements.—*Philip L. Oglesby and George Dickson, National Bureau of Standards, Washington, D. C.* Several factors affecting the accuracy of color determinations of silicate cements were investigated. Data were obtained with a Gardner automatic color difference meter. Initial results indicated that if reproducible values were to be obtained, the color calibration standards normally used with the meter should be replaced with standards more uniform over the surface and more nearly similar in color, translucency, and size to the cement specimens. Large differences in color values were obtained when the medium surrounding the standards was changed from air to water. Best results for standards with established values in air appear to be obtained from comparison of the standards in air with the cement specimens in water. Studies of the effect of temperature on the aging changes of silicate specimens indicated that some materials which came to color equilibrium in about 3 weeks at 25° C. reached equilibrium in approximately 1 week at 37° C. Measurement of the colors of a number of commercially available cements indicated that a system of standard shades suitable for specification use could be developed on the basis of requirements for the tristimulus values for Y (reflectance) and Z (yellowness).

M12. Investigations to Reduce the Solubility of Silicate Cements.—*Frank J. Brauer (USN), Eli E. White, Jr., and Claire L. Burns, National*

Bureau of Standards, Washington, D. C. The material leached out on storing silicate cements in water was analyzed spectrochemically. The major constituents of the leach are sodium (over 20 per cent), phosphorus (10-20 per cent), aluminum (5-6 per cent), silicone (4-5 per cent), and calcium (1-2 per cent). Various chemicals such as methoxyphenylacetic acid, carbethoxyalkylsilanes, alkyltriethoxysilane and silicone R-23 were added to silicate cement mixes in an effort to reduce the solubility and disintegration of the hardened product. Incorporation of small amounts of silicone R-23 into the mixes reduced the solubility and disintegration by about 20 per cent on storage in water for 23 hours and 7 days, respectively. Setting time was not affected by addition of silicone, but the compressive strength was often lowered. The added silicone apparently combines with the free silica in the mix. but does not react with the sodium to form an insoluble product.

M13. Synthesis of a Silica-Resin Direct Filling Material: Progress Report.—*R. L. Bowen, American Dental Association Research Division, National Bureau of Standards, Washington, D. C.* An epoxy resin (Epon 828), polymerized by ionic addition with orthophosphoric acid, was adversely affected by water. However, the addition product of methacrylic acid and Epon 828 was a liquid that would polymerize at room temperature by a free radical mechanism. The reaction product of bisphenol A and glycidyl methacrylate, thinned with tetraethyleneglycol dimethacrylate and activated with dimethyl-para-toluidene, hardened at room temperature in about 3 minutes when mixed with fused silica powder containing benzoyl peroxide. This tooth-colored solid had a compressive strength of 11,000 psi (A.D.A. Spec. No. 9). A vinylsilane treatment of the silica increased the compressive strength to 16,000 psi. The optical opacity was about $35_{C_{0.70}}$. The solubility and disintegration was 0.25 per cent. When part of the fused silica was replaced with colorless flint glass (refractive index 1.5795), the x-ray opacity was between that of enamel and dentin without visual difference in color or opacity. When 5 parts benzoyl peroxide per 100 parts resin was used with 74 to 83 per cent vinylsilane-treated silica, the compressive strength averaged 22,000 psi. It was less without the silica. Further investigation and development of this approach to an improved direct filling material is definitely indicated.

M14. An Electromechanical Dental Model Surveyor.—*Donald C. Hudson, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.* An instrument designed to improve the accuracy of dental model surveying procedures has been developed. Mechanical design makes use of adjustable ball bearings in the hinge of the surveyor arm and in the vertical spindle carriage. Free motion of arm and surveyor tool are assured and take-up of play in any direction is easily accomplished. Conventional metal undercut gauges and marking styli are accommodated; no ink or graphite rod marking is employed. Minimal contact of marking tool and model is all that is required. There is no danger of abrading the model or of obtaining broad markings through wear of the tool. An electric current is employed to achieve the marks by which heights of contour or undercut areas may be outlined. The marking tool is made negative with respect to the cast and current is controlled by means of a foot switch. Momentary switch closure is required to produce a sharp legible mark—no mark is produced except as current is permitted to flow. An indicator solution is applied to the damp cast in areas to be surveyed. Cationic attraction at the point of tool-model contact, with formation of hydroxides and change in pH, causes a color change. Marks are fugitive (10-15 minutes) but ample time is afforded the operator to properly outline clasps or other design features with suitable marking pencils.

M15. A Study of the Surface Reaction of Enamel to Various Polishing Agents.—*E. W. Skinner, P. L. Copeland, L. J. Fiegel, and R. G. Earnshaw, Northwestern University and Illinois Institute of Technology, Chicago.* It is generally conceded that the polishing action is related to the appearance of a microcrystalline surface layer as evidenced by the change in the electron diffraction pattern from the usual ring pattern in the unpolished surface to the halo pattern in the polished surface. Specimens of tooth enamel have been subjected to the action of various dentifrice polishing agents under constant pressure, rotational speed, and time of polishing, and the electron diffraction patterns of the resulting surfaces studied. Halo patterns were found with some of the agents. Others failed to produce perceptible surface changes, whereas some resulted in partial polishing. In one instance, the enamel surface became impregnated with the agent.

M16. The Microhardness of Enamel and Dentin.—*R. G. Craig and F. A. Peyton, University of Michigan, School of Dentistry, Ann Arbor.* The hardness of tooth structures has been determined by a variety of methods including abrasion, scratch, and indentation technics. These investigations have led to generalizations concerning the variation in the hardness of tooth structures which are at times in variance with each other. This preliminary study of the microhardness of tooth structures was undertaken in an attempt to establish any trends existing in the hardness of enamel and dentin from various areas of the same tooth and from different types of teeth. The microhardness of cross-sections of 26 mature, freshly extracted teeth was determined by using a Knoop diamond indenter. The measurements were made on sections at known distances from the occlusal surface or incisal edge, and were made in a direction from buccal or labial to lingual, or from mesial to distal. In one experiment the hardness measurements were taken around the tooth at a definite distance from the enamel surface or dentin-enamel junction. The range of Knoop hardness values for enamel and dentin was 272-380 and 60-76, the average values being 335 ± 25 and 69 ± 4, respectively. The deviation in the hardness values from tooth to tooth and from section to section of a tooth, as well as differences in any one section, obscured any trends that may have existed in the microhardness of the enamel or dentin. Additional study is required to determine the cause of these differences before any definite statements can be made about the hardness variations in tooth structures.

M17. Design Factors of Dental Burs as Related to Cutting Effectiveness.—*D. J. Rigas, E. W. Skinner, R. S. Lindenmeyer, and R. L. Lasater, Northwestern University, Chicago.* The relation of certain design factors in dental burs to cutting speed in tusk ivory has been investigated. It has been found that cross cutting of the blade, a positive rake angle on the cutting edge, and a reduction in the number of teeth of the bur tend to increase the cutting effectiveness. Other factors, such as spirality, balance, run out and higher rotational speeds (circa 100,000 r.p.m.) are being studied at the present time. Although it is admitted that the conclusions obtained are strictly applicable only for the conditions of the experiment, it can be reasoned that the effects of these design variables can be applied qualitatively to the cutting of other materials, such as tooth enamel.

M18. A Tachometer for the Evaluation of Rotary Cutting Speeds.—*James R. Richards, Biophysics Laboratory, Naval Medical Research Institute, and Arne G. Nielsen, U. S. Naval Dental School, National Naval Medical Center, Bethesda.* A tachometer for the evaluation of rotary cutting speeds has been developed and a prototype constructed as a result of the combined efforts of

the Naval Medical Research Institute and the Naval Dental School. With this device it is possible to produce an accurate, continuous record of the variable rotary speed of cutting instruments under actual operating conditions. The tachometer will measure speeds ranging from 50 to 500,000 r.p.m. The principle of the dynamo is utilized in the operation of the instrument. A light, permanent, doughnut-shaped magnet, placed on the shaft of a rotating cutting instrument, is rotated within a magnetic field produced by a coil mounted on the handpiece. The electromagnetic force (E.M.F.) generated by the rotating magnet, which is directly proportional to its rotating speed, is amplified and modified to provide the means for the continuous, accurate measurement of rotary instrument speeds. Rotary speeds may be observed on an oscilloscope or recorded continuously with a pen and ink recorder. It is expected that this instrument will provide a valuable adjunct for an accurate evaluation of rotary cutting procedures.

M19. The Measurement of Torque-Speed Characteristics of Dental Handpieces.—*Duane F. Taylor, John W. Kumpula and R. R. Perkins, National Bureau of Standards, Washington, D. C.* An apparatus has been developed for studying the torque-speed characteristics of high speed dental handpieces. The equipment has separate systems for measuring the rotational speeds of the motor and handpiece, and for applying and measuring the torque delivered to the instrument shaft. Speeds are measured to an uncertainty of approximately 2 per cent by means of photocell-audio-frequency meter combinations. Torque is applied to a disk in the handpiece by a pendulum-mounted electromagnet, the combination acting as an eddy-current brake. The torque measuring system is capable of determining applied torques to 1 per cent over the range of 0-400 gram-centimeters. Torque-speed curves have been obtained for typical designs of ball-bearing, multiplying, and turbine handpieces. The free running speeds of the handpieces studied ranged from 20,000 to more than 200,000 r.p.m. The maximum delivered torques ranged from 20 to 300 gram-centimeters.

M20. The Effect of Residual Stress on the Deformation of Hydrocolloid Impression Materials.—*H. Wm. Gilmore, Ralph W. Phillips, and Marjorie L. Swartz, Indiana University School of Dentistry, Indianapolis.* Water change, measured quantitatively, in reversible hydrocolloid impressions when stored in various media had been reported previously. This investigation included the irreversible hydrocolloids as well as isolating the effect of residual stress, per se, upon the dimensional stability of both reversible and irreversible hydrocolloids. Stress was induced by varying the gelation time and by use of undercut dies. Dimensional change was measured by use of a master control casting seated back on the stone die. The actual water change of irreversible hydrocolloid in various media compared favorably to the values which had been obtained for reversible hydrocolloid, the smallest change (and often minute) being in 100 per cent relative humidity. Distortion on storage of reversible and irreversible hydrocolloid impressions was greater whenever the residual stress was increased, even though the water change was the same as the control. The data indicate that syneresis or imbibition is not the sole factor which influences dimensional stability. The effect of storage temperature is also discussed.

M21. Determination of the Shrinkage of Elastic Dental Impression Materials.—*Eugene J. Molnar, Coe Laboratories, Inc.* Since ideal conditions for determination of shrinkage do not exist, an empirical method was devised for arriving at the measurement of the shrinkage in elastic dental impression

materials. Cylindrical specimens (1 inch in length and 1 inch in diameter) were provided with an aluminum rider attached to the top of each specimen. The change in length was read on a Vernier height gauge from the deviation of the rider's hairline from its original alignment with the gauge marker. This determination was carried out on a cast iron surface plate. The 2 rubber-like materials (Thiokol and silicone types), the plasticized and gelled acrylic types, and the agar-agar and alginate types comprise the scope of material tested. The unconfined specimens were exposed to the air. The method employed and the comparative data obtained cover the time range and shrinkage from initial set of the materials through several weeks of exposure of the specimens.

M22. Clinical and Physical Studies of the Silicone Rubber Impression Materials.—*G. E. Myers, University of Michigan, School of Dentistry, Ann Arbor.* A variety of silicone polymers have been utilized as the basis of compounded impression materials. No one of these possesses all the desirable clinical properties required for an elastic impression material. Advantages which all the silicones offer over the Thiokol materials are more esthetic coloring, easier mixing, absence of odor, and the materials do not stain linen or clothing. Disadvantages vary from one material to another and include the following: values for percentage strain and percentage permanent deformation too high, formation of gas during polymerization with the production of stone dies with surface porosity, surface tack, and the silicones do not electroform as readily as the Thiokols. Initial and final setting of the silicones can be modified by varying the concentration of the catalyst and by the use of retarders. Available products give a range of initial setting times from 1.5 minutes to 8.5 minutes, with final setting times varying from 3.5 minutes to 11.5 minutes at room temperature. An increase of room temperature from 70° F. to 80° F. accelerated the initial set of a typical silicone product by 0.5 minute. A comparable Thiokol product was accelerated 4.0 minutes by the same temperature change. When tested by a modification of the consistency test of No. 8 A.D.A. Specification, a typical tray material had a value of 21 mm. and a typical syringe material a value of 28 mm.

M23. Silicone Rubber as Dental Impression Materials.—*R. R. McGregor, A. D. Chipman, and R. R. Maneri, Dow Corning Corporation, Midland, Mich.* Silicone pastes as a base for dental impression materials may be prepared which when mixed with suitable catalysts vulcanize to rubbers at room temperature in 3 to 10 minutes, the time being governed by the composition of the silicone and the nature and amount of catalyst. The pastes may be prepared in viscosities suitable for syringe or tray application. Rubbery impression materials so prepared have good dimensional stability which is retained under atmospheric conditions for a period of a week or longer, eliminating the necessity for rapid stone duplication and allowing work to be accomplished at leisure. Materials suitable for dental impressions are two-component systems, generally consisting of a basic paste which contributes the rubbery mass and a liquid or compounded catalyst. By variation of fillers, additives, and catalysts, a wide variety of properties can be obtained to determine handling characteristics, speed of set, hardness, and color, among others. These materials may be dispersed from tubes and mixed in a conventional manner. A wide variety of silicone rubbers is available, each of which may have some desirable as well as undesirable characteristics. Early problems of stone porosity caused by gas evolution have been eliminated.

M24. A Clinical Evaluation of Modern Instrumentation for the Removal of Tooth Structure.—*Jack L. Hartley, School of Aviation Medicine,*

USAF, Randolph Air Force Base, Texas. A study was made of the relative cutting effectiveness and "annoyance factors" of 4 newer types of cutting instruments. Electronic timers, activated by the foot control of these instruments, recorded the time of actual cutting. Over-all operative time, including hand instrumentation, was also recorded. All preparations were accomplished by the same operator. The instruments evaluated were: (1) an ultrasonic machine, (2) a hydraulically operated machine, rotating at 45,000 r.p.m., (3) a belt-driven handpiece, rotating at 125,000 r.p.m., and (4) a conventional "high speed" instrument operated at 12,000 r.p.m. One hundred and fifty cavity preparations were accomplished for each cutting method. The "annoyance factor" was determined by a comparison of constant level tape recordings of tooth-transmitted bone conducted vibrations of the skull during cutting by the four methods. The least annoying instrument was determined to be the ultrasonic machine. The most annoying was the conventional handpiece rotating at 12,000 r.p.m. with both carbide and diamond bars.

M25. Modern Methods of Tooth Structure Removal. A Symposium.—Moderator: *P. B. Taylor.* Participants: *J. L. Bernier, U. S. Army, J. V. Borden, Washington, D. C., J. L. Hartley, U. S. Air Force, J. J. Kennedy, National Institutes of Health, R. A. McEwen, Atlanta, Ga., A. G. Nielsen, U. S. Navy, and H. M. Tanner, University of Southern California, Los Angeles.* The participants will present a résumé of modern methods of tooth structure removal. Instruments of various types will be discussed and evaluated. The effects of the current armamentarium on the tooth, the patient and the operator will be analyzed. Considerable biological and physiologic data will be presented. Extensive time is allocated for discussion of questions from the floor.

M26. Changes in Intercast Dimensions Produced by Mounting Procedures.—*Robert R. Perkins and Merrill G. Wheatcroft, U. S. Naval Dental School, National Naval Medical Center, Bethesda.* A method has been devised whereby continuous measurement of changes in vertical intercast distance can be made during mounting procedures. Most methods of measuring dimensional change of gypsum products have required some lapse of time between mixing and recording of dimensional change. Consequently the early phenomena of changes during setting were unobserved. The apparatus consists of a displacement gauge based on the differential transformer principle. The coils are fixed in the mandibular cast and the assembly is mounted on the articulator prior to the test. The maxillary cast holds the core of the transformer and the sensitivity of its movement can be adjusted to 5 microns. The results showed that when a dehydrated stone cast was mounted with plaster of Paris or stone, an initial increase in intercast space occurred. This was followed by a closure of the intercast space which could be correlated with the exothermic reaction. A decreased amount of opening occurred when the cast was coated with wax or petroleum jelly. When the cast was thoroughly soaked prior to mounting, the initial opening was not observed. Therefore, when the cast was dry, the initial opening was balanced by the subsequent closure, and the final vertical change was less than when hydrated casts were mounted.

M27. The Casting Shrinkage of Cobalt-Chromium Alloys.—*R. G. Earnshaw, Northwestern University, Chicago, and University of Manchester (England).* A study has been made of the casting shrinkage of the dental cobalt-chromium alloys. These alloys have an inherent casting shrinkage (this is, unrestricted thermal contraction from solidus to room temperature) of about 3 per cent. Direct measurements, made on rods 0.125 in. in diameter by about 2.5 in. long, cast into a cold mold, have shown that as a group the dental

cobalt-chromium alloys have an actual casting shrinkage of about 2.2 per cent, which requires a mold expansion of 2.25 per cent for accurate compensation. It was also found that the shape of the casting and the compressive strength of the investment are 2 interrelated factors which can affect the actual casting shrinkage.

M28. Some Effects of Cavity Roughness on Adaptation of Gold Castings.—*G. T. Charbeneau, School of Dentistry, University of Michigan, Ann Arbor.* The effect of certain variables of the casting technic upon the roughness of the casting has been established by using the Proficorder. The inherent casting roughness varies primarily with the type of investment. Cross-sectioned gold castings, seated both with and without cement into preparations on Ivorine blocks, show that smoother walls result in better wall and margin adaptation than do rougher walls. The degree of roughness as well as the type of roughness appears to have some significance in the ability of the casting to seat well. The presence of cement affects the ability of the Class I and full crown casting to be seated in the manner used in this study. This effect was not noted with Class II and VI castings. It was found that better adaptation of the lateral walls is obtained for the full crown casting, when using a hygroscopic technic, where a softer wax is used to form the occlusal surface and proximal angles, while using a harder wax to form the proximal walls.

M29. An Evaluation of Indirect Inlay Pattern Waxes and Their Manipulation.—*George E. Miller and David B. Mahler, University of Oregon Dental School, Portland.* This investigation is concerned with the evaluation of pattern waxes and their manipulation as used in the indirect gold casting procedure. Little work has been done with respect to the effect of such variables as type of wax (soft vs. hard) or manipulation technic (pressure used; temperature of wax, die, and environment; procedure of building the pattern). It is the purpose of this investigation to determine these effects on the accuracy of the resulting wax pattern. A test procedure for measuring accuracy was devised using a master MOD die in which pyramidal indentations were made and from which measurements could be taken. This is essentially a progress report in which the test procedure is evaluated and the results of some preliminary tests described.

M30. Micro Studies of Some Dental Casting Golds. Part II.—*Clyde E. Ingersoll, Williams Gold Refining Co., Inc., Buffalo, N. Y.* Investigation has been continuing on the problem of inclusions in gold alloys. After many tests, a pattern asserted itself, leading to the identification of the elements causing the inclusions. Three elements acting together cause the inclusions. These elements are gold, copper, and carbon. Removal of any one of the three results in an inclusion-free alloy. Not all alloys containing these three elements have inclusions, however. The inclusions may be avoided by the addition of certain carbon stabilizing elements to the alloy. If additives are not used, the alloys subject to inclusion formation should be manufactured in a carbon-free procedure, and when melts are made for casting, graphite crucibles and graphite- or charcoal-containing fluxes should be avoided.

M31. Further Evidence on the Hygroscopic Expansion of Dental Gypsum Materials.—*David B. Mahler and Richard E. Moffitt, University of Oregon Dental School, Portland.* Recent evidence has supported the presumption that the hygroscopic expansion of dental gypsum materials is essentially setting expansion as produced by gypsum crystals growing from supersaturated solutions. If this premise is valid, the precise definition of hygroscopic expansion

depends exclusively upon defining the role of the water supplied to the setting material. It was theorized that the decreasing water phase in the setting expansion produces a compressive force on the growing crystals, thereby inhibiting their growth. Water supplied to the material during setting increases the water phase thereby decreasing the compressive force and allowing for greater expansion. A similar effect should be produced by reducing the surface tension of the mix water (without supplying any additional water during setting) since this would reduce the compressive force on the particles. Wetting agents to reduce surface tension were added to the mix water and expansions higher than normal setting expansion were recorded. A correlation between the increased setting expansions and the surface tension of the mix water was demonstrated, thus lending support to the presumptions indicated above.

M32. Method for Evaluation of Dimensional Changes in Dental Bridge Soldering.—*Thomas R. Anderson, Carl W. Fairhurst, and Gunnar Ryge, Marquette University, Milwaukee.* In order to facilitate study of dimensional change and warpage as related to gap distance, shape and surface condition of soldering surfaces, and temperature, a modified dental bridge assembly was designed and constructed. A master model was fabricated with modified full crown preparations reproduced in brass and spaced 8 mm. apart. An assembly consisting of three full crowns with connecting gauge rods parallel to the facial and occlusal surfaces at a distance of ¾ inch and width connector rods to each crown was cast in a type B gold. Threaded holes were made at the contact areas of the crowns and interchangeable gold screws were cast to fit the threaded holes. Solder junctions were established between the heads of the gold screws. Alignment facets were ground on the gauge rods after which they were cut between the crowns to allow movement of the parts during investment, preheating, and soldering. Linear dimensional changes of the assembly were measured at the location of the cuts of the gauge rods by means of thickness gauges at various stages of the soldering procedure, and warpage was observed by checking the alignment of the facets on the gauge rods.

M33. Autoradiographs of Solder Joints With Fluorine-18.—*Saul M. Bien and Herbert D. Ayers, Jr., Columbia University, New York.* Continuing previous investigations into the nature of the union of solder with rustless metals, the addition of fluorine-18 was made to the flux. Fluorine-18 was supplied as MgF_2 with an activity of approximately 3.5 mc. in 100 mg. of the product. This was spatulated into 10 Gm. commercial fluoride flux on a parchment pad. Forty-two samples of 18/8 stainless steel, type 304 (diameter 1.3 mm.), were soldered with this flux using silver-copper-zinc-nickel solder. The samples were mounted vertically in 6 acrylic blocks, 7 samples to the block. The cross section of each solder joint was polished with 6 grades of metallurgical paper. Five mounts were placed on film (ASA160) for 21 hours. One mount was placed on extrafast periapical x-ray film for 16 hours. All films were developed in x-ray developer for 5 minutes. Examination of the film showed an aureolus of increased opacity around a transparent core, measuring 1.3 mm. in diameter. On certain of the autoradiographs the areas of increased opacity would appear to correspond in shape and area to the area of solder in contact with the film. On one autoradiograph there appeared to be a transparent core encircled by a narrow band of increased opacity which was encircled by a transparent zone, which was in turn encircled by an opaque area for one third of its circumference. Autoradiographs were not obtained using concentrations of 0.65 mc. or 1.5 mc. in 10 Gm. of flux.

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BACTERIAL ENZYMES AND PERIODONTAL DISEASE

MARGARET R. DEWAR*

Institute of Dental Research, United Dental Hospital, Sydney, Australia

THE etiology of most periodontal disease is usually considered to be very complex and many causes, both local and systemic, have been implicated. The local factors produce conditions favorable for bacterial growth, particularly anaerobic conditions.

Even if bacteria are not the prime cause of periodontal disease, at least they have a claim to consideration as a contributory cause, inasmuch as they may be responsible for accentuating or accelerating tissue breakdown. Hence, a study of the enzyme systems of oral bacteria found in gingival debris, insofar as these may be capable of destroying the appropriate tissue elements, could be of value.

The present paper is concerned with an investigation of the presence of carbohydrases and proteinases in bacteria present in gingival debris taken from individuals with normal gingivae and those suffering from periodontal disease.

MATERIALS

Gingival Debris.—(a) 38 samples of gingival debris, unfiltered; (b) 17 samples of gingival debris, Seitz filtered; (c) 10 samples of gingival debris, filtered through No. 5 sintered-glass filters ([b] and [c] from individuals with periodontitis); (d) 12 cultures of gingival debris in gentian violet-serum-nutrient broth and 5 cultures from guinea pigs treated in accordance with the Rosebury technic[1]; and (e) 8 samples of gingival debris prepared as acetone powder, ground with Carborundum, extracted with M/15 phosphate buffers[2] at pH 5.5 and 7.4, and filtered through a No. 5 sintered-glass filter. All filtered material was tested for sterility before and after examination for enzyme activity.

Salivary Debris.—Saliva (15 ml.), stimulated by the chewing of paraffin wax, was collected from each of 12 individuals with normal gingivae. The saliva was left for one hour to allow the mucoid to liquefy and was then centrifuged. The supernatant was discarded and the deposit resuspended in 3 ml. Ringer's solution.

Pure Cultures of Bacteria.—Pure cultures of fusiforms (in serum-nutrient broth) and spirochetes (in Hampp medium), isolated from extracted teeth, were obtained and tested under aerobic and anaerobic conditions.

METHODS

The periodontal tissues of each patient were examined with probe and mirror and by x-ray. Each individual was then classified as having (1) normal gingivae, (2) marginal gingivitis, (3) marginal gingivitis with bone loss, (4)

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periodontitis, or (5) necrotizing ulcerative gingivitis. As much calculus, materia alba, and general debris as possible were removed from around the teeth, from the gingivae, and from periodontal pockets and suspended in 3 ml. Ringer's solution.

Detection of Enzymes.—

The samples of material were tested for the presence of enzymes by the following technics:

A. *Carbohydrases:*

1. *Hyaluronidase* was detected by the A.C.R.A. technic of Burnet,[3] which measures the depolymerizing enzyme. Hyaluronic acid prepared from umbilical cord[4, 5] was used as substrate (composition: nitrogen 2.97 per cent, glucosamine 28.2 per cent, sulfur 0.15 per cent). Incubation of the substrate (15 mg./ml.) in M/15 phosphate at pH 5.5 with an equal volume of enzyme solution for 18 hours at 37° C. was found to be a sensitive method, capable of demonstrating the presence of less than 0.1 Turbidity Reducing Unit (Wyeth) and less than 1 Benger Unit of the enzyme. In some instances the presence of the enzyme was also demonstrated by the detection of glucosamine liberated from the substrate. This technic reveals the presence of the hydrolyzing in addition to the depolymerizing enzymes.

2. *Chondroitin sulfatase* was detected by Boas' modification[6] of the Morgan-Elson technic for measuring the glucosamine liberated from chondroitin sulfate. The substrate used was chondroitin sulfate prepared from ox trachea[7] (composition: nitrogen 2.36 per cent, glucosamine 22.8 per cent, sulfur 6.4 per cent). It was free of hyaluronic acid, no glucosamine being liberated by pneumococcus. The most sensitive method devised for the detection of this enzyme was to incubate equal volumes of enzyme solution and substrate (25 mg./ml.) in M/15 phosphate at pH 7.4 at 37° C. for 4 days. Even so, sensitivity was not high, the detection of the enzyme in 24-hour cultures of Proteus being only possible in dilutions of less than 1 in 20. This method was valid for the detection of chondroitin sulfatase in the presence of hyaluronidase. This was shown by dilution, the hyaluronidase in the gingival debris being 10 to 500 times more active than the chondroitin sulfatase. The hyaluronidase was more sensitive also to inhibition by serum, being inhibited 40 to 80 per cent, while chondroitin sulfatase was inhibited only 15 to 20 per cent.

B. *Proteinases:* Because there is a series of "collagenases" which can attack collagen in its various physical states, their specificity depending on the nature of the substrate, particular attention was given to the substrate. As the acid conditions of inflammation and antigen-antibody reaction[8] are sufficient to alter collagen partially, different collagen substrates (gelatin, hide powder, and native collagen) were used in the following experiments.

1. *Gelatinase* was estimated by the technic of Evans and Wardlaw.[9] Gelatin, 2.5 per cent, in M/15 phosphate (pH 7.4) was incubated for 18 hours at 37° C. with an equal volume of enzyme solution. By this technic the enzyme could be detected in a 1 in 160 dilution of a 48-hour broth culture of *Staphylococcus pyogenes.*

2. *Hide-powder collagenase* was detected by the liberation of color from azocoll (acid- and heat-treated collagen).[10] Approximately 10 mg. sterile azocoll in 0.5 ml. M/15 phosphate (pH 7.4) were incubated with an equal volume of enzyme solution for 7 days at 37° C. Sensitivity was high, the enzyme being detected in a 1 in 2,000 dilution of a 24-hour broth culture of *Clostridium histolyticum*.

3. *Collagenase*. Native collagen was prepared by dissection of tendons from ox tail, washing them for 3 minutes each with water, acetone, and ether, and then sterilizing by exposing small pieces (about 15 mg.) to 3 per cent hydrogen peroxide for 7 minutes. Small portions were brought to constant dry weight by heating 18 hours at 75° C. Such a preparation of collagen was resistant to trypsin but extension of the time taken to wash or to sterilize it increased its susceptibility to attack by trypsin. Collagenase activity was detected by the loss in dry weight of small portions of collagen (about 15 mg.) after incubation at 37° C. for 7 days in 0.5 ml. M/15 phosphate at pH 7.4 and 0.5 ml. enzyme solution. In some experiments, activity was also measured by determining the amount of increase in total nitrogen and hydroxyproline in the solution. By these technics, enzyme activity was detected in a 1 in 2,000 dilution of a 24-hour broth culture of *Clostridium histolyticum*.

Filtration.—

Filtration was carried out through No. 5 sintered-glass filters (pore diameter less than 1μ) and Seitz filters (2.9 cm. diameter, grade D_9). Tests carried out with filtrates of pure cultures or preparations of the enzymes in M/15 phosphate buffers (pH 6.0 to 7.4) showed that no detectable loss of activity of enzyme occurred as a result of filtration through Seitz or glass filters, despite claims by Russell and Sherwood[11] that great loss of activity of hyaluronidase occurred on filtering through Seitz pads.

RESULTS AND DISCUSSION

1. *Unfiltered Gingival Debris.*—It can be seen from Table I that hyaluronidase, chondroitin sulfatase, and gelatinase were present in all unfiltered debris tested. Native collagenase was present in the debris removed from the gingivae of persons suffering, respectively, from periodontitis and gingivitis, and those having clinically normal mouths, in the ratio of 3:2:0.

Schultz-Haudt and Scherp[12] have isolated, from gingival debris, organisms capable of producing chondroitin sulfatase. They[13] have also detected an enzyme capable of hydrolyzing native collagen in cultures from gingival debris.

The significance of the failure to detect the presence of a native collagenase in the majority of the samples of debris removed from patients suffering from necrotizing ulcerative gingivitis is not clear because, during the progress of this disease, rapid destruction of the collagen of the gingiva occurs and is apparent to the naked eye. However, inflammation and general tissue reaction are extreme in these cases, so that an alteration in the collagen possibly makes it susceptible to a greater number of enzymes.

TABLE I
ENZYME ACTIVITY OF UNFILTERED GINGIVAL DEBRIS REMOVED FROM INDIVIDUALS WITH VARIOUS CONDITIONS OF THE PERIODONTAL TISSUES

	CLINICAL DIAGNOSIS														
	NORMAL			MARGINAL GINGIVITIS			MARGINAL GINGIVITIS †BONE LOSS		PERIODONTITIS			NECROTIZING ULCERATIVE GINGIVITIS		
ENZYMES	+*	±	–	+	±	–	+	–	+	±	–	+	±	–
Hyaluronidase	1	2	0	8		0	4	0	16		0	7		0
Chondroitin sulfatase	–	–	–	4		0	4	0	16		0	7		0
Gelatinase	3	0	0	8		0	4	0	16		0	7		0
Hide-powder collagenase	1	1	1	3	2	3	4	0	15		1	7		0
Native collagenase	0	0	3	1	2	5	2	2	4	5	6	1	1	5

*+, ±, and – represent the degree of enzyme activity: positive, trace, and negative.

2. *Filtered Gingival Debris.*—In filtered material, enzymes were detected infrequently (Table II). Therefore, some of the enzymes in the gingival debris either will not pass through the filter or pass through in such small quantities that they cannot be detected by present methods.

TABLE II
ENZYME ACTIVITY OF FILTERED GINGIVAL DEBRIS FROM INDIVIDUALS WITH PERIODONTITIS

	GINGIVAL DEBRIS					
	SEITZ FILTERED			SINTERED-GLASS FILTERED		
ENZYMES	+*	±	–	+	±	–
Hyaluronidase, suspended in						
a. Ringer's solution	4	2	11	2	0	3
b. Water + gelatin + pyrophosphate	–	–	–	5	0	0
Chondroitin sulfatase	0	1	9	0	0	3
Gelatinase	2	1	14	2	1	1
Hide-powder collagenase	1	0	13	2	0	2
Native collagenase	0	0	10	0	0	2

*+, ±, and – represent the degree of enzyme activity: positive, trace, and negative.

When cultures from the unfiltered debris were prepared, the enzymes were detected after filtration. Seitz filtrates of cultures from gingival debris in gentian violet-serum-nutrient broth and in exudate from guinea pigs were rich in the enzymes. Hyaluronidase, chondroitin sulfatase, and gelatinase were detected in all broth filtrates. Hide-powder collagenase was detected in 6 of the 12 and traces of native collagenase in 3. All enzymes except native collagenase were detected in the filtrates of the guinea pig exudate in moderate concentrations. Only small traces of native collagenase were detected. It would seem, then, that those enzymes in unfiltered debris and in the cultures from the debris are possibly not all exocellular enzymes excreted during growth of the bacteria but are mainly endocellular enzymes freed on autolysis. All cultures tested were incubated several days and autolysis could have taken place. The presence of these filtrable endocellular enzymes was demonstrated in extracts of acetone powder of the debris which had been ground with Carborundum; in such extracts, all enzymes except native collagenase were detected consistently.

These endocellular enzymes are important, because once an opening in the gingivae occurs (either as a result of the action of exocellular hyaluronidase trauma, inflammation, or ulceration), bacteria enter the tissues.[14] Thus, in periodontitis, gram-positive and gram-negative bacteria can be demonstrated many cell layers below the epithelium. Also, rupture of the bacteria may occur on the gingival surface due to death by aging or changes in pH or osmotic pressure. The enzymes are then freed to penetrate the tissue.

Schultz-Haudt, Bruce, and Bibby[15] detected a collagenase capable of destroying the hide powder in the filtrate (using sintered-glass filters) of 8 out of 10 samples of gingival debris taken from the mouths of individuals suffering from gingivitis. This prevalence was greater than that found in the present work despite the fact that Schultz-Haudt, Bruce, and Bibby used a shorter incubation period for the detection of the enzymes. The presence of hyaluronidase was detected with approximately the same frequency in the two investigations.

3. *Salivary Debris.*—The presence of collagenase capable of destroying hide powder was investigated by Lucas and Thonard[16] in bacteria isolated from 12 persons suffering from periodontal disease and 6 persons with healthy gingivae. They isolated 56 such strains, 17 of which were from healthy mouths. The presence, in healthy mouths, of bacteria possessing such tissue-destroying enzymes is supported by the present work on salivary sediment. Such enzymes were present in the saliva from all 12 persons tested, except hide-powder collagenase which was absent from one sample of saliva and native collagenase which was absent from 2 and only present in trace quantities in 6 samples. Therefore, it seems quite feasible that, when predisposing factors are present in the mouth which allow the bacteria to multiply, high concentrations of the enzymes may develop. These cause tissue destruction and gingival disease results.

4. *Pure Cultures of Bacteria.*—Pure cultures of fusiforms and spirochetes showed only slight proteolytic activity, irrespective of whether they were tested under aerobic or anaerobic conditions. In the 10 fusiform cultures tested, collagenases were not detected and hyaluronidase, chondroitin sulfatase, and gelatinase were detected only once. In the 20 spirochete cultures a proportionate degree of enzymic activity was observed.

These organisms are considered by some workers[17, 18] to be possible causative agents in some gingival diseases and it was thought that their enzyme pattern might be such that they could readily destroy connective tissue. The results are therefore surprising. However, only pure cultures were tested and it may be that these organisms produce the more active enzymes symbiotically, as Schultz-Haudt and Scherp[19] have shown for phenol sulfatase production by fusiforms. In view of the negative results obtained in the present investigation, the work of McDonald, Sutton, Knoll, and Madlener,[20] is of interest because they have produced experimental "fuso-spirochaetal" infections with 4 strains of organisms, among which fusiforms and spirochetes were not included.

The importance of tissue-destroying enzymes in the gingival and periodontal pocket, whether such enzymes originate from bacteria or from the tissues, is at

present difficult to evaluate. For example, there are present in the plasma antihyaluronidase factors[21] but, although in vitro these substances can inhibit hyaluronidase, their role in vivo is not clear. Also present in the tissue itself are collagenases active in the catabolic processes of metabolism. Gersh and Catchpole[22] have claimed that they are present in increased concentration in inflammation and disease. It is not yet known, however, what activates these tissue enzymes.

Shroff[23] suggested that enzymes (collagenases) are not necessary for the destruction of periodontal fibers, because "toxic substances" from inflammatory zones may cause swelling and breaking of collagen fibers and prevent maintenance of osteoblastic and cementoblastic activity. However, the action of such toxic substances of inflammatory origin does not automatically exclude the bacterial enzymes from a part in the tissue destruction; on the contrary it emphasizes the importance of the inflammatory reaction and its causation—mainly local factors, including bacteria.

CONCLUSION

There are tissue-destroying enzymes of bacterial origin present in material from around the teeth. The importance of tissue-destroying enzymes is at present difficult to evaluate.

SUMMARY

1. Gingival debris was collected from 38 individuals with various degrees of gingival and periodontal disease.

2. Filtered and unfiltered material was tested for the presence of tissue-destroying enzymes: hyaluronidase, chondroitin sulfatase, gelatinase, and two collagenases, one able to attack hide powder and the other native collagen.

3. In the unfiltered material, hyaluronidase, chondroitin sulfatase, and gelatinase were always present. Hide-powder collagenase was present in most samples and native collagenase was present in the ratio of 0:2:3, respectively, in normal individuals, those with marginal gingivitis and those with periodontitis.

4. In the filtered material the enzymes were detected only infrequently.

5. Pure cultures of spirochetes and fusiforms showed only slight enzyme activity.

6. Salivary sediment contained all the enzymes, except 1 sample from which hide-powder collagenase was absent and 2 samples from which native collagenase was absent.

7. The significance of the presence of these enzymes in the gingival pocket and in the saliva is discussed.

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THE HYALURONIDASE ACTIVITY OF SALIVA

I. The Determination of Characteristics of Hyaluronidase Activity in Young Male Adults

G. H. ROVELSTAD, D.D.S., M.S.D., J. H. GELLER, B.S., M.S., AND A. H. COHEN, B.S., D.D.S.

The Dental Research Laboratory, Dental Department, Administrative Command, U. S. Naval Training Center, Bainbridge, Md.

ENZYME activity of saliva has been widely studied. Some recent reports[1-3] have demonstrated the enzyme spectrum of saliva and suggested that a variability exists in certain enzymes that may exhibit a cause and effect relationship to diseases of both hard and soft tissues.

It is the purpose of this project to study one of these enzymes, hyaluronidase, in order to determine its activity in saliva and its possible effects on the hard and soft tissues of the oral cavity.

The specific objective of this project shall be to establish base line data on hyaluronidase in saliva of an average group of young male adults.

REVIEW OF LITERATURE

Hyaluronidase* has been described as a mucolytic enzyme which depolymerizes and hydrolyzes the intercellular cementing substance known as hyaluronic acid.[4] Since this action permits the entrance and spread of bacteria,[4, 5] tumors,[6, 7] dyes and other materials,[8] the enzyme has been called a "spreading factor."[4]

Spermatozoa are the largest single source of hyaluronidase in the body.[9, 10] The enzyme from this source functions by dissolving the hyaluronic acid gel surrounding the ovum, thus allowing for penetration by the sperm cells.[11, 12] In addition, hyaluronidase has been found in the spleen, ciliary body, testes, snake and insect venoms, as well as in the products of certain microorganisms.[13-20] The invasiveness of these microorganisms is known to be directly related to the concentration of hyaluronidase produced by them.[20, 21] The enzyme has, also, been identified in saliva where it is thought to be of bacterial origin.[1-3]

Hyaluronic acid, the substrate for hyaluronidase, has been isolated from vitreous humor, umbilical cord, synovial fluid, skin, and some tumors of mesodermal fluid.[22-25] There has also been evidence obtained by staining tooth structure that indicates the presence of a calcium-hyaluronate-protein complex in the dentin.[26]

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*Or hyaluronidases, term used to include several hyaluronidases.

The literature offers several excellent reviews on the sources, properties, and clinical aspects of hyaluronic acid and hyaluronidase.[8, 27-31]

In the hyaluronidase determination, the mucin clot prevention test is most convenient for screening purposes, but for quantitative results either the turbidometric test or the viscosity-reduction test is required.[30-33] The viscometric technic for the determination of hyaluronidase activity has been described by Hadidian and Pirie.[34] Lisanti has modified this procedure for the purpose of evaluating the hyaluronidase activity of saliva.[1] This technic has been further modified for this study.

MATERIALS AND METHODS

A. *Substrate.*—The substrate for the viscosity reduction test of hyaluronidase is a known concentration of hyaluronic acid extracted from human umbilical cords and preserved in chloroform.*

The dialyzed hyaluronic acid is then mixed according to the following proportions: 24 mg. of hyaluronic acid, 6 ml. of 0.5M phosphate buffer (45.36 Gm. KH_2PO_4, 89.36 gr. Na_2HPO_4, $7H_2O$. Make up 2,000 ml., adjust pH to 7.0) and distilled water to make up a volume of 32 ml. Thus, each milliliter of the substrate solution contains 0.75 mg. hyaluronic acid. Two milliliters of this solution plus 0.4 ml. of 0.2 M borate mixture (10 ml. saline, 10 ml. 0.05 M $MgCl_2$, 20 ml. 0.2 M borate buffer [24.8 Gm. H_3BO_3, 99.0 ml. 2N NaOH, H_2O to make up 2,000 ml. adjusted to pH 6.8 with 2N HCl]) plus 0.1 ml. of saline make up the total substrate as it is placed in the viscosimeter. Two and one-half milliliters of saliva is then added to the viscosimeter so that the total volume of the mixture is 5 ml. This gives a solution buffered to an approximate pH of optimum enzyme activity (6.6). The substrate is freshly prepared for use each time.

B. *Sample.*—Twenty milliliters of saliva is obtained by paraffin stimulation from the patient (after 5 hours of supervised fasting, during which time no liquids or solids are allowed to enter the oral cavity and smoking is prohibited). The saliva is collected in a stoppered container, resting in cracked ice, and is kept refrigerated until used. Fifteen milliliters of this saliva is centrifuged at 1,500 G. for 10 minutes at room temperature. Two and one-half milliliters of the supernatant saliva is then placed in a sterile tube, stoppered, and stored at 4° C. The hyaluronidase activity determinations are carried out between 18 and 24 hours after the collection.

C. *Technic.*—Two and one-half milliliters of hyaluronic acid substrate is added to 2.5 ml. of saliva in an Ostwald-Fenske Viscosimeter. The viscosimeter is maintained in a constant temperature water bath at 37° C. throughout the test period. The initial flow time of the mixture is determined as soon as possible and repeated after 45 minutes. The difference between the initial and the final readings is recorded in seconds and is used as the index of enzyme activity. In order to study a number of cases simultaneously, a series of viscosimeters are used, each having been standardized under test conditions to one instrument.

*Obtained from Tufts College of Medicine and Dentistry, Boston, Mass.

D. *Evaluation of Technic.*—Laboratory evaluations of the viscosity-reduction technic have demonstrated that it is possible to study as many as 40 different samples simultaneously. The enzyme determinations are accurate to a plus or minus 0.6 seconds for known concentrations of commercial preparations of hyaluronidase and to a plus or minus 0.8 seconds for different aliquots of a given sample of saliva. The technical error of this procedure, therefore, is 1.6 seconds and should be considered in all determinations. The activity of this enzyme in saliva is not altered by the test period of 45 minutes at 37° C.; neither does a variation in storage time at 4° C., within a plus or minus 8 hours, show any effect. At 37° C., the viscosity of the substrate is stable for at least 6 hours and the viscosity of saliva is stable for at least 2 hours.

RESULTS

A. Twenty milliliters of saliva was collected under supervision from one young adult at 8 o'clock in the morning. The subject had not eaten breakfast nor had he brushed his teeth prior to this collection. Twenty milliliters of saliva was collected again 1 hour later and every hour for the next 5 hours. All the saliva samples collected were fasting samples except the 4- and 6-hour specimens. The enzyme activities for these different samples are recorded in Table I.

TABLE I
HYALURONIDASE ACTIVITY OF SALIVA OF ONE YOUNG MALE ADULT OVER A PERIOD OF 6 HOURS
(USNTC, BAINBRIDGE, MD., 1954)

TIME SAMPLE COLLECTED	TIME LAPSE (IN HOURS) BETWEEN SAMPLES	HYALURONIDASE ACTIVITY*
8 A.M.	–	10.7
9 A.M.	1	8.3
10 A.M.	2	8.6
11 A.M.	3	8.2
12 M.	4	13.4
2 P.M.	6	12.7
Average		10.3

*In seconds drop in viscosity of substrate.

The hyaluronidase activity of saliva of one individual ranged from 8.2 to 13.4 seconds activity within 6 hours of the initial tests with an over-all average of 10.3 seconds. The highest reading of activity was recorded at 12 M., 20 minutes after the consumption of food. The reading is of interest inasmuch as the first 4 determinations were made on fasting samples of saliva. Two additional tests were repeated at 27 hours and at 51 hours from the time of the original collection using fasting saliva and gave the same results as the original fasting sample (10.7 and 10.4 seconds, respectively).

B. Twenty milliliters of saliva was collected under supervision from 3 young male adults at 11 o'clock in the morning after 5 hours of supervised fasting. The hyaluronidase activity was then determined for each sample. These procedures were repeated once a week for 6 weeks. The enzyme activities of these subjects are recorded in Table II. The first subject (Case No. 281) showed a variability of not more than ±3.1 seconds from an average activity of

14.5 seconds, the range being from 12.5 to 17.6 seconds; the second subject (Case No. 282) showed a variability of not more than ±4.4 seconds from an average activity of 15.2 seconds, the range being from 11.5 seconds to 19.6 seconds; while the third subject (Case No. 285) showed a variability of not more than ±5.9 seconds from an average activity of 24.1 seconds, the range being from 20.9 seconds to 29.9 seconds.

TABLE II
HYALURONIDASE ACTIVITY OF SALIVA OF 3 YOUNG MALE ADULTS AT 11 O'CLOCK IN THE MORNING OVER A PERIOD OF 6 WEEKS
(USNTC, BAINBRIDGE, MD., 1954)

CASE NUMBER	HYALURONIDASE ACTIVITY*							
	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	6TH WEEK	AVERAGE	VARIABILITY
281	12.5	13.1	14.3	17.6	13.5	16.0	14.5	± 3.1
282	11.5	18.8	12.8	16.5	11.8	19.6	15.2	± 4.4
285	29.9	22.6	23.9	21.6	20.9	25.7	24.1	± 5.9

*In seconds drop in viscosity of substrate.

The average range for the 6-week period was 7.4 seconds while the average maximum variability from the mean was 4.5 seconds. The average difference between each week's test results for Case No. 281 was 2.34 seconds, for Case No. 282 was 5.90 seconds, and for Case No. 285 was 3.28 seconds; the average for the group being 3.84 seconds.

C. Twenty milliliters of saliva was collected under supervision from each member of a company of naval recruits at 5 o'clock in the morning after 8 hours of unsupervised fasting. These samples were analyzed for hyaluronidase activity. This procedure was repeated during the subsequent week so that 2 determinations were completed on each recruit at an interval of 1 week. The enzyme activities of this group are summarized in Table III.

TABLE III
SUMMARY OF HYALURONIDASE ACTIVITY OF SALIVA OF 42 YOUNG MALE ADULTS AT 5 O'CLOCK IN THE MORNING AFTER 8 HOURS OF UNSUPERVISED FASTING AND AT 11 O'CLOCK IN THE MORNING AFTER 5 HOURS OF SUPERVISED FASTING
(USNTC, BAINBRIDGE, MD., 1954)

	HYALURONIDASE ACTIVITY*					
	5 A.M.			11 A.M.		
	1ST WEEK	2ND WEEK	MEAN	1ST WEEK	2ND WEEK	MEAN
Mean	16.9	15.4	16.3	12.8	11.3	12.0
Range	11.8	11.8	11.2	15.3	16.8	14.5
Standard deviation	2.36	2.31	2.08	3.73	4.23	3.63

*In seconds drop in viscosity of substrate.

On the same days as above, 20 ml. of saliva was collected under supervision from each member of the same company of naval recruits at 11 o'clock in the morning after 5 hours of supervised fasting. These samples of saliva were analyzed for hyaluronidase activity. This procedure was repeated during the subsequent week so that 2 hyaluronidase activities were recorded on each recruit at an interval of one week. These enzyme activities are summarized in Table III.

It will be noted that the average enzyme activity at 5 A.M. was 16.3 seconds, whereas the average enzyme activity at 11 A.M. was 12.0 seconds. The range and the standard deviation were greater for the 11 A.M. samples than for the 5 A.M. samples. The average difference and maximum differences were greater, however, between samples of saliva collected at 5 A.M. and 11 A.M. on the same day than on samples collected at the same time of the day (5 A.M. or 11 A.M.) on consecutive weeks.

D. A sample of saliva was collected from each of 77 young male adults at 11 A.M. after 5 hours of supervised fasting and the hyaluronidase activity of each sample was determined. These procedures were repeated once a week on 3 consecutive weeks (Table IV). The average enzyme activity of the group was 11.5 seconds for the first, 10.0 seconds for the second, and 10.2 seconds for the third week's samples. The average activity for all samples was 10.55 seconds, with a highest recorded activity of 18.4 seconds and a lowest recorded activity of 1.5 seconds.

TABLE IV
HYALURONIDASE ACTIVITY OF SALIVA OVER A 3-WEEK PERIOD OF 72 YOUNG MALE ADULTS AT 11 O'CLOCK IN THE MORNING AFTER 5 HOURS OF SUPERVISED FASTING (USNTC, BAINBRIDGE, MD., 1954)

	HYALURONIDASE ACTIVITY*			
	1ST WEEK	2ND WEEK	3RD WEEK	AVERAGE
Mean	11.5	10.0	10.2	10.5
Range	16.2	16.9	15.7	12.4
Standard deviation	3.64	4.03	3.73	3.16

*In seconds drop in viscosity of substrate.

Selecting the average of the 3 tests as the indication of the relative activity of each case, the frequency distribution of the averages for each case results in a normal curve. The standard deviation of these data is 3.16. Thirty-four per cent of the average results fell below 5 seconds activity and 27 per cent above 15 seconds activity.

DISCUSSION

The hyaluronidase activity of saliva has been determined for a number of young male adults under varying circumstances and times using the viscosity-reduction technic. This activity has been found to be variable at different periods of one day as well as on different days. This variability, however, appears to stay within certain classified limits of enzyme activity (high or low) over a period of weeks. The analysis of the hyaluronidase activities of salivas of a large group of young adults, collected over a period of 3 weeks, demonstrates a relative constancy of hyaluronidase activity of saliva, collectively, as well as individually. From these tests, base line data for the determination of the relationship of the hyaluronidase activity of saliva to dental diseases are set up.

CONCLUSIONS

1. The hyaluronidase activity of human saliva is variable from hour to hour, as well as from day to day.

2. In spite of the obvious variation, the hyaluronidase activity for one individual will remain relatively constant in a high, medium, or low range of activity over a period of weeks.

3. The hyaluronidase activity of fasting saliva collected from young male adults early in the morning is greater than that collected later in the day.

4. The most reproducible results for the hyaluronidase activity of saliva can be obtained by using fasting samples of saliva collected at the same time of the day under the same circumstances, rather than at different times of the day.

5. The average hyaluronidase activity (in seconds decrease in viscosity of hyaluronic acid substrate for a period of 45 minutes at 37° C.) of fasting saliva of young male adults at 11 o'clock in the morning is 10.5 seconds according to the viscosity-reduction technic. The range is from 1.5 seconds to 18.5 seconds and the standard deviation is 3.16. These results are based on the average of 3 tests carried out on each individual at weekly intervals (77 in the study group).

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THE HYALURONIDASE ACTIVITY OF SALIVA

II. The Relationship of Hyaluronidase Activity to Dental Caries Experience, Gingivitis, and Oral Hygiene in Young Male Adults

G. H. ROVELSTAD, D.D.S., M.S.D., J. H. GELLER, B.S., M.S., AND A. H. COHEN, B.S., D.D.S.

The Dental Research Laboratory, Dental Department, Administrative Command, U.S. Naval Training Center, Bainbridge, Md.

THE hyaluronidase activity of saliva has been evaluated for young adults and has been found to be relatively constant as well as reproducible within a given range of activity for the individual.[1] It is the purpose of this study to determine the relationship of the hyaluronidase activity of saliva to the dental caries experience, gingivitis, and oral hygiene of young adults.

MATERIALS AND METHODS

The subjects for this study were naval recruits undergoing training at the U.S. Naval Training Center, Bainbridge, Md., from January, 1955, to February, 1956. Both random and purposive selection of individuals were used in order to obtain a test group with an adequate distribution of dental conditions for analysis.

Saliva was collected by paraffin stimulation from naval recruits at 11 o'clock in the morning, after 5 hours of supervised fasting, and analyzed for hyaluronidase activity. A 20 ml. sample of saliva was taken from each subject at weekly intervals for a period of 3 weeks. The hyaluronidase activity determinations were completed on each sample using the viscosity-reduction technic previously described.[1] All results were recorded in seconds decrease in viscosity of a saliva-hyaluronic acid substrate mixture. The median test result was selected for each case and recorded for correlation.

The test group consisted of 1,049 subjects, 75 of which had no carious teeth at the time of the test. The latter group included 63 individuals who showed no evidence of dental caries or dental caries experience. One hundred and two subjects had marked and advanced changes of the gingival tissues at the time of the tests; 798 of the test group had a total of 26 or more teeth and were used for the oral hygiene evaluation.

An oral examination using a mouth mirror, explorer, and adequate lighting, supported by bite-wing radiographs interpreted with the aid of a magnifying glass (10×), was the method used for diagnosing dental caries. The

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number of DMF (decayed, missing, or filled) teeth, the number of carious teeth, and the number of carious surfaces were tabulated on each case. Characteristics of the study group are shown in Table I.

An oral color photograph was taken on each case. This was accomplished with a fixed focus 35 mm. clinical camera, using an electronic stroboscopic light.* One view of each case was taken using lip retractors, with the teeth and oral mucosa fully visible. Gingivitis and oral hygiene were determined by the use of these photographs since it provided a means of rating the degree of gingival involvement and the cleanliness of the teeth on a comparative basis. The method of diagnosis and interpretation of these photographs follows.

Fifty or more 2 × 2 color transparencies were placed on a viewing box with artificial lighting. One examiner viewed each case separately with magnification and classified the subject according to the condition of the gingival tissue adjacent to the 6 anterior maxillary and mandibular teeth for gingivitis and according to the cleanliness of the anterior maxillary and mandibular teeth and surrounding area.

Five classifications of gingivitis were set up as follows:

0. *No gingivitis.*—Gingivae appear normal in color in relation to the other surrounding mucosal tissues of the patient, the contour is normal and unbroken, the interdental papilla is neither swollen nor flattened, and there is no evidence of edema of tissues.

1. *Possible gingivitis.*—Probable but unpronounced change in any of the aforementioned criteria.

2. *Definite gingivitis.*—Slight but evident change in contour, interdental papilla is swollen and inflamed, and there is evidence of edema.

3. *Moderate gingivitis.*—Definite and substantial change in any of the aforementioned criteria.

4. *Severe gingivitis.*—Marked and advanced changes in any of the aforementioned criteria.

Characteristics of the study group are shown in Table I.

Three classifications of oral hygiene were set up as follows: (1) *good oral hygiene* was considered to exist when all tooth surfaces were clean and free of debris, stains may or may not have been present but, if present, did not change the surface contour or texture of the teeth; the interdental spaces also appeared clean; (2) *fair oral hygiene* existed when there was some debris on some tooth surfaces and in some interdental spaces; stains may or may not have been present and, if present, exhibited changes of surface contour; (3) *poor oral hygiene* was present when much debris appeared on most tooth surfaces and interdental spaces; stains were frequent and a soft pulpy type of residue was obvious.

*The photographic equipment used for the study was originally designed and tested by B. L. Taylor and C. A. Schlack of the Naval Medical Research Institute, Bethesda, Md., and further modified by H. W. Lyon as reported in Research Report, Project NM 008 012.03.04, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md., May 27, 1953, vol. 11, pp. 693-714.

TABLE I
CHARACTERISTICS OF STUDY GROUP OF 1,049 YOUNG MALE ADULTS FOR EVALUATION OF HYALURONIDASE ACTIVITY OF SALIVA IN RELATION TO DENTAL CARIES EXPERIENCE AND GINGIVITIS (USNTC, BAINBRIDGE, MD., 1955-1956)

ITEM	AVERAGE	RANGE
Age	18.5	17-25
Dental experience		
DMF (3rd molars excluded)	13.5	0-28
Carious teeth	8.8	0-32
Teeth present	26.6	0-32
Gingivitis*	2.0	0-4
Hyaluronidase activity†	10.8	0.5-24.9

*0—None, 1—Possible, 2—Probable, 3—Moderate, 4—Severe.
†In seconds drop in viscosity.

Table II shows the characteristics of 798 subjects used in the evaluation of oral hygiene.

TABLE II
SUMMARY OF STUDY GROUP OF 798 YOUNG MALE ADULTS WITH 26 OR MORE TEETH FOR EVALUATION OF HYALURONIDASE OF SALIVA IN RELATION TO ORAL HYGIENE (USNTC, BAINBRIDGE, MD., 1955-1956)

ITEM	AVERAGE	RANGE
Age	18.5	17-25
Number of teeth present	28.2	26-32
Oral hygiene*	2	1-3
Hyaluronidase activity†	10.8	0.5-24.9

*1—Good, 2—Fair, 3—Poor.
†In seconds drop in viscosity.

RESULTS

The average hyaluronidase activity of the saliva of this group of 1,049 young adults (used to evaluate dental caries experience and gingivitis) was found to be 10.8 seconds decrease in the viscosity of saliva-substrate mixture. The range of activity extended from 0.5 to 24.9 seconds (Table I). The standard deviation for the data was 4.1 seconds. The average hyaluronidase activity of saliva, range of activity, and standard deviation of the group used in the oral hygiene determination was found to be similar to the entire group (Table III).

TABLE III
HYALURONIDASE ACTIVITY* OF 1,049 NAVAL RECRUITS ACCORDING TO QUANTITATIVE GROUPS WITHIN THE VARIOUS METHODS OF CLASSIFYING CARIES EXPERIENCE (USNTC, BAINBRIDGE, MD., 1955-1956)

	ALL GROUPS	GROUP				
		0	1-4	5-9	10-19	20-32
	Average Viscosity Drop in Seconds					
DMF teeth	10.8	11.3	11.3	11.4	10.7	10.4
Carious teeth	10.8	10.7	11.1	11.6	10.9	10.6
Carious surfaces	10.8	10.7	11.3	10.8	10.7	10.9

*Median result of three separate determinations.

The distribution of results of the mean hyaluronidase activity of saliva according to the various classifications of DMF teeth, number of carious teeth, and number of carious surfaces is summarized in Table III. It will be noted

that the average for all cases of 10.8 seconds is similar to that of all groups classified whether of high or low caries experience groups. Analysis of the data indicated no significant difference between the high and low caries experience groups.

The average hyaluronidase activity found in each of the 5 classifications of gingivitis are presented in Table IV. It will be noted that the group having a severe gingivitis gave an average hyaluronidase activity of 13.1 seconds as compared to 9.8 seconds for the gingivitis-free group and 10.8 seconds for the total study group.

TABLE IV
HYALURONIDASE ACTIVITY* OF 1,049 NAVAL RECRUITS ACCORDING TO THE VARIOUS GROUPS OF GINGIVITIS (USNTC, BAINBRIDGE, MD., 1955-1956)

	ALL CASES	GINGIVITIS				
		NONE	POSSIBLE	PROBABLE	MODERATE	SEVERE
		Average Viscosity Drop in Seconds				
Activity	10.8	9.8	10.5	11.0	12.0	13.1

*Median result of three separate determinations.

The average hyaluronidase activity found in each of the classifications of oral hygiene are presented in Table V. It will be noted that the group having a "poor oral hygiene" presented an average hyaluronidase activity of 12.3 seconds as compared to 10.4 seconds for the "good oral hygiene" group, and 10.8 seconds for the total study group.

TABLE V
HYALURONIDASE ACTIVITY* OF 798 NAVAL RECRUITS ACCORDING TO THE VARIOUS GROUPS OF ORAL HYGIENE (USNTC, BAINBRIDGE, MD., 1955-1956)

	ALL CASES	ORAL HYGIENE		
		GOOD	FAIR	POOR
		Average Viscosity Drop in Seconds		
Activity	10.8	10.4	11.2	12.3

*Median result of three separate determinations.

DISCUSSION

Regardless of the method of rating dental caries experience for this group of patients, whether total past experience (DMF) or presence of the actual lesions at the time of the test (carious teeth), there is no evidence of any relationship to the hyaluronidase activity of saliva. Inasmuch as a comparatively large number of cases, free from dental caries, were available and included within the study, and inasmuch as all determinations were carried out under identical conditions, the end results should be representative data for young adults.

Gingivitis was diagnosed and classified from oral color photographs. In the study group (being young patients), there was a majority of cases of either minimal or no apparent change from the normal in the soft tissues surrounding the anterior teeth. Nevertheless, there were over 100 cases in which marked and advanced changes from the normal were present at the time of these tests. Therefore, a wide range of soft tissue conditions were apparent

in the test group. A positive relationship is indicated by the distribution of cases according to the hyaluronidase activity of saliva and the degree of gingivitis of the cases; the greater the hyaluronidase activity, the greater the gingivitis. The average difference between the groups has been found to be statistically significant.

Oral hygiene was diagnosed and classified from oral color photographs. These photographs were taken after the chewing of paraffin for saliva stimulation. The cleanliness of the teeth and interdental spaces was thus interpreted. An even distribution in the classes of oral hygiene was apparent in the test group. A positive relationship is indicated by the distribution of cases according to hyaluronidase activity of saliva and oral hygiene; the poorer the oral hygiene the greater the hyaluronidase activity. The average difference between the groups has been found to be statistically significant.

CONCLUSION

From these data it may be concluded that hyaluronidase activity of saliva of young naval recruits as indicated by the viscosity-reduction technic carried out on 3 samples of saliva, collected at weekly intervals at 11 o'clock in the morning after 5 hours of supervised fasting, has no relationship to dental caries experience as revealed by the number of DMF teeth, the number of carious teeth, or the number of carious surfaces at the time of the tests.

However, hyaluronidase activity of saliva is related to gingivitis and oral hygiene as diagnosed and classified by oral color photographs.

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A HISTOCHEMICAL STUDY OF THE SALIVARY GLANDS IN NORMAL AND CORTISONE-INJECTED WHITE MICE

GERALD SHKLAR, IRVING GLICKMAN, AND SAMUEL TURESKY

Department of Oral Pathology, Tufts University School of Dental Medicine, Boston, Mass.

ALTERATIONS in the major salivary glands of the rat and mouse have been noted in the alarm reaction[1, 2] and in animals injected with cortisone.[3] Using routine fixation and staining with hematoxylin-eosin a slight diminution in the interacinar connective tissue has been noted. It was felt that histochemical technics might provide further information regarding the effects of systemically administered cortisone upon the major salivary glands. An experiment was undertaken for this purpose using the periodic acid-Schiff (PAS) technic for demonstrating mucopolysaccharides and mucoproteins.[4-8]

EXPERIMENTAL PROCEDURE

Fifty white mice, R.A.P. strain (25 male and 25 female), approximately 6 weeks of age and weighing 18 to 24 Gm., were divided into 2 groups of 25 animals. Group I received daily subcutaneous injections of 0.25 mg. cortisone acetate (Cortone-Acetate, Merck). One group of the control group (Group 2) received daily subcutaneous injections of physiologic saline and the remaining animals were uninjected. In all cases, littermates were distributed between experimental and control groups. The animals were sacrificed at the end of a 35-day experimental period. The parotid, submaxillary, and sublingual glands were removed in a block dissection and the internal organs were removed. The tissues were fixed in a 10 per cent solution of formalin and 80 per cent alcohol. Serial sections were stained with hematoxylin-eosin and by the periodic-acid Schiff method.

OBSERVATIONS

Control Animals.—The parotid gland in the mouse is composed of serous cells which present a darkly staining nucleus and granular cytoplasm when stained with hematoxylin and eosin (Fig. 1). With the periodic-acid Schiff (PAS) method the cells stain faintly, with the nuclei and cell membrane appearing slightly darker (Fig. 2).

The sublingual gland is composed of mucous acini with a flattened darkly staining nucleus and clear cytoplasm (Fig. 3). With the PAS method the cells

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stain deeply. PAS positive material appears prominently at the base of the cells in the area of the nucleus, in the cell membrane, and in strands which divide the cytoplasm into irregularly arranged spherical spaces. Some of the spaces are filled with pale staining material, others are partially or completely devoid of stain (Fig. 4). The nucleus does not stand out, in contrast to the remainder of the cells, as it does in hemotoxylin and eosin stained sections. The ducts stain faintly, but cellular outlines and basement membrane can be discerned.

The structure of the submaxillary gland of the mouse is unique in that the exact nature of acinar and tubular elements has not been definitely established. The acinar portion resembles serous cells, with oval darkly staining nuclei and granular cytoplasm, but it has been suggested that they function somewhat differently.[2] For this reason they have been referred to as "special serous cells."[9] Tubules lined with columnar epithelium, with flattened nuclei at the base, form an extensive ductal system of the glands (Fig. 5). Because the ducts resemble mucous acini it has been suggested that they may also function as secretory cells.[10] The serous acini and ducts both contain PAS positive material with the former staining somewhat more intensely. In the serous cells, the nuclei are prominent. The cytoplasm has a globular configuration, with deeply staining septa. The cytoplasm of the ductal cells is uniformly granular. The nucleus stains slightly darker (Fig. 6).

A sex difference has been described in the morphology of the submaxillary gland ducts in mice beginning with the seventh week[10, 11] but consistent differences could not be detected in our material.

PAS sections of all salivary glands were treated with diastase to determine whether any of the stained material was glycogen. Liver sections were used as controls. This treatment did not alter the staining of the salivary glands, in contrast with the disappearance of stained material in the liver.

Experimental Animals.—The most notable difference between the control and cortisone-injected animals was observed in the PAS stained sections of the submaxillary glands. This was manifested as an increase in the intensity of staining of the serous cells. The staining of the ductal cells was unaltered. As a result the contrast between the acinar and ductal cells in the cortisone-injected animals was greater than in the controls (Figs. 7 and 8).

In the sublingual glands, there appeared to be an increase in the staining of the mucous cells in the cortisone-injected animals. However, because of the intensity of staining in both the control and experimental animals it was difficult to establish the presence of a real difference. No changes were noted in the PAS stained sections of the parotid glands of the cortisone-injected animals.

Treatment of PAS sections with diastase did not modify the staining quality in the cortisone-injected animals. In the hematoxylin and eosin sections there was a slight diminution in the interacinar connective tissue in all of the salivary glands of the cortisone-injected animals.

Fig. 1.

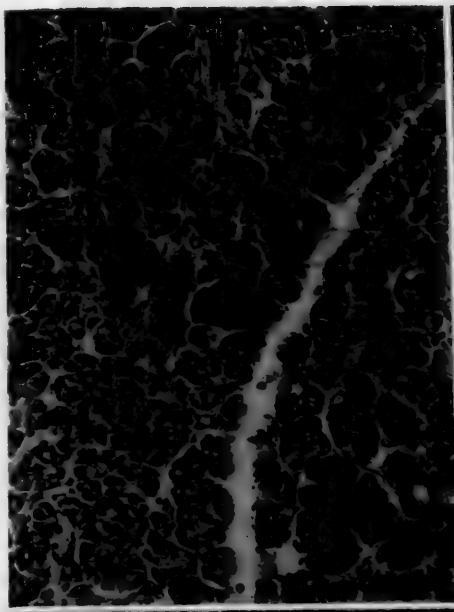

Fig. 2.

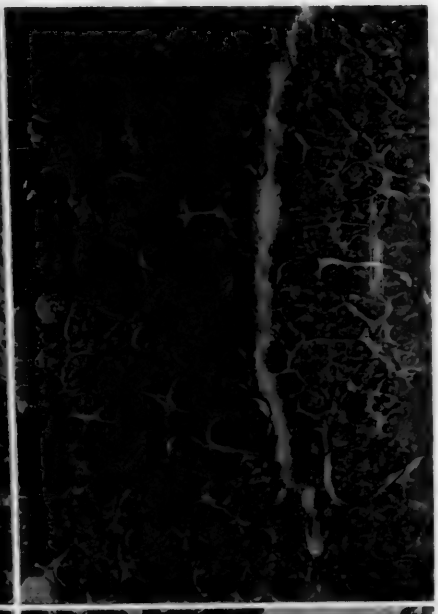

Fig. 3.

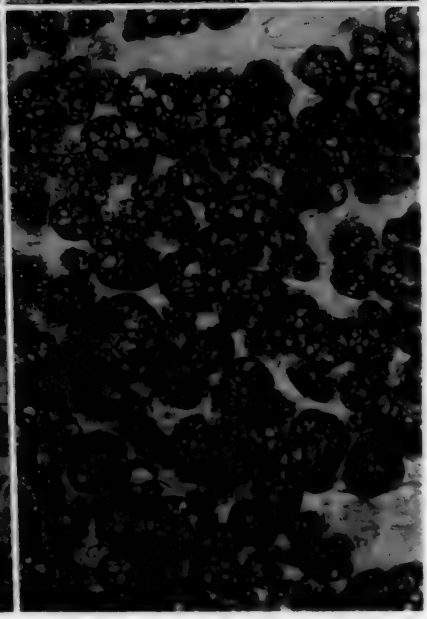

Fig. 4.

Fig. 1.—Parotid gland in control animal. Note the serous acini with darkly staining nuclei and granular cytoplasm. (Hematoxylin-eosin; orig. mag. ×210.)

Fig. 2.—Parotid gland in control animal. The cells stain faintly but the nuclei and cellular membranes can be discerned. (Periodic acid-Schiff; orig. mag. ×210.)

Fig. 3.—Sublingual gland in control animal. Note the mucous acini with flattened nuclei, and relatively clear cytoplasm. (Hematoxylin-eosin; orig. mag. ×210.)

Fig. 4.—Sublingual gland in control animal. Note the deep staining of the acinar cells. The dark staining material is prominent in nuclei and cell membranes. (Periodic acid-Schiff; orig. mag. ×210.)

Fig. 5.

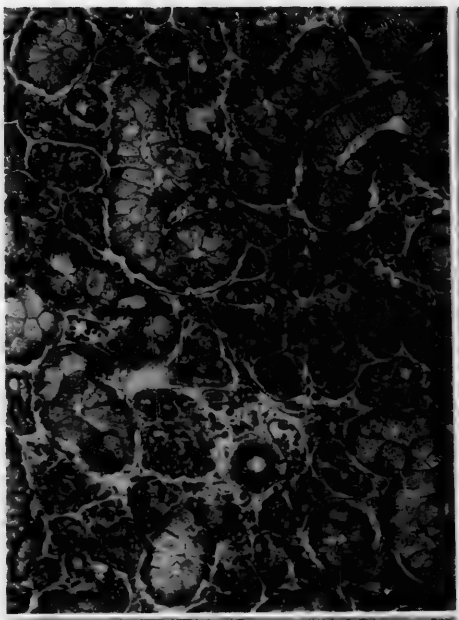

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 5.—Submaxillary gland in control animal. Note the acinar and ductal portions. The acini resemble the serous acini of the parotid gland with their darkly staining nuclei and granular cytoplasm. The ducts resemble mucous acini. (Hematoxylin-eosin; orig. mag. ×210.)

Fig. 6.—Submaxillary gland in control animal. The acinar portion stains slightly darker than the adjacent ducts. (Periodic acid-Schiff; orig. mag. ×210.)

Fig. 7.—Submaxillary gland in control animal. (Periodic acid-Schiff; orig. mag. ×210.)

Fig. 8.—Submaxillary gland in cortisone-injected animal. Note the increased intensity of stain of the acini. The contrast between the tubular and acinar elements is more pronounced than in the controls (compare with Fig. 7). (Periodic acid-Schiff; orig. mag. ×210.)

DISCUSSION

Staining with the PAS method indicates the distribution of water-insoluble, alcohol-insoluble polysaccharides. Treating sections with diastase revealed that the material which stained in the salivary glands of the control and experimental animals was not glycogen.

The sublingual gland stained heavily for mucopolysaccharides, while the parotid gland took very little stain. This was to be expected because of the mucous nature of the sublingual secretion.[13-15]

In the submaxillary gland, the acinar portion morphologically resembled the serous cells of the parotid, but stained more deeply. In this respect, there was some resemblance to the more darkly staining mucous cells of the sublingual gland. Based upon staining qualities there may be some validity in the suggestion that the serous acini of the submaxillary gland of the mouse function somewhat differently than the serous acini of the parotid.

The major change attributable to the systemic administration of cortisone was manifested as an increase in the staining of the serous cells of the submaxillary gland. This may be interpreted as an increased cellular content of water-insoluble, alcohol-insoluble polysaccharides or an increased availability of reactive groups caused by depolymerization of normally existent polysaccharides. The treatment with diastase indicated that the increased staining in the cortisone-injected animals could not be attributed to the presence of glycogen.

The assumption that the increased staining in the acinar cells of the submaxillary gland represents an increase in polysaccharide material would be consistent with the reported increase in systemic carbohydrate availability caused by cortisone.[12] Using this line of reasoning, it could be hypothesized that there was an increased production and storage of mucous material because of the increased availability of carbohydrate. Had it been possible for us to demonstrate clear-cut differences between the mucous glands of experimental and control animals it would have lent support to this hypothesis. However, the normal mucous glands stained so deeply with PAS that anything short of a pronounced increase in polysaccharides is obscured.

Although our findings indicate that the systemic administration of cortisone resulted in an increase in the staining of polysaccharides in acinar epithelium of the submaxillary gland, they do not provide a basis for explaining the manner in which this change was brought about.

CONCLUSION

The microscopic structure and distribution of polysaccharides were described in the salivary glands of young adult male and female white mice using alcohol-formalin fixation and hematoxylin-eosin and periodic acid-Schiff staining methods.

Systemic administration of cortisone resulted in an increased intensity in the staining of polysaccharides in the acinar epithelium of the submaxillary gland.

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THE PROTRUSIVE MOVEMENT IN ARTICULATORS

A. A. D. DERKSEN AND W. VAN HAERINGEN

Dental School of the University of Utrecht and Physical Laboratory of the University of Utrecht, Utrecht, Netherlands

AN articulator is an apparatus with which it is possible to reproduce the spatial relation between the jaws mutually, as well as with respect to the temporomandibular joint, and with which we are able to imitate approximately the individual chewing movements. To determine whether an articulator really imitates the human masticatory apparatus or not, it is necessary to know the movements of the lower jaw with regard to the upper jaw. Only those articulators that have a joint and an incisal pin are discussed here and only the protrusive movements of the human jaw and the articulators are discussed.

The protrusive movement is in planes parallel to the sagittal plane. The median sagittal plane is a plane of symmetry of the lower jaw while, for practical reasons, the occlusal plane is supposed to be horizontal. The condylar path, as well as the incisal path, are assumed to be straight lines in the temporomandibular joint as well as in an articulator. Almost all articulators are based on this principle.

According to Bergström,[5] Benson demonstrated that the movements of an articulator are not the same when the joint slots are in the upper part as when they are in the lower part. Only articulators in which the joint slots were attached to the upper part possessed the possibility of giving a fairly acceptable result. On the other hand, Bergström stated that the joints of an articulator should have their shaft or corresponding elements placed on the lower part and their paths on their upper part.

Articulators with a movable upper member, with a joint and an incisal pin, can be subdivided in the types I^b, II^b, III^b, IV^b (Fig. 1). When the lower member is movable, there are theoretically the possibilities of types I^a, II^a, III^a, IV^a (Fig. 1).

In these 8 types, the plane of drawing is sagittal. In all diagrams, the straight line c is the intersecting line from the sagittal plane and the plane through the condyle paths. In the same way, the straight line i is the intersecting line of sagittal plane and the incisal table. It is assumed that both right and left condyle guides are symmetrical with regard to the median sagittal plane. As the median sagittal plane is a plane of symmetry with regard to both the members, it is sufficient to describe the protrusive movements as movements of the sagittal projections of several points. The analysis of the protrusive

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movements may thus be simplified by the description of their projections in the sagittal plane. Thus, the protrusive movement is reduced to the relative movement of 2 planes in the sagittal plane.

For example: in diagram I^a the lower plane (lower bow) moves with respect to the upper plane (upper bow) as follows: point C moves along the straight line c, point I moves along the straight line i. The distance between C and I remains constant. When the upper bow is movable instead of the lower one, it will be clear from diagram I^b that the relative movement of a certain point from the latter bow with regard to the former, is essentially the same. It is also clear that the types II^a and II^b, III^a and III^b, IV^a and IV^b, respectively, are identical in the same way. This means that it will be sufficient to investigate the types I^a,

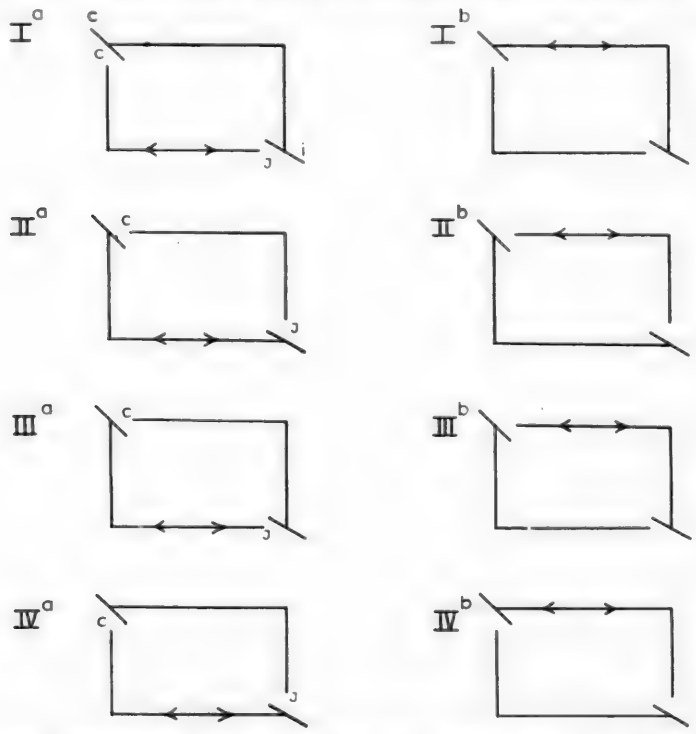

Fig. 1.—Moving principles of the 8 types of articulators with a joint and an incisal pin. The arrows indicate the movable member.

II^a, III^a, and IV^a. In this respect, the following points are of importance: (1) the angle formed by the 2 straight lines c and i and (2) the beginning position of C and I and the distance between them. It should be noted that in types III^a and IV^a the distance between C and I is not constant.

For a comparative study of the types I^a – IV^a, we start from a certain position, in which C and I are situated in a certain way and in which c and i form a certain angle. This given situation will be considered as the successive starting point for the movements I^a, II^a, III^a, and IV^a.

The Dentatus articulator ARH (type II[b]) is shown in Fig. 2. Fig. 3 shows a schematic representation of it projected in the median sagittal plane.

The measurements on the Dentatus articulator gave the following results: CB = 30 mm., BA = 24 mm., AI = 115 mm. The models are mounted in the articulator, according to the triangle of Bonwill, i.e., CS is the perpendicular in this equilateral triangle, with sides of 100 mm.

Fig. 2.—The Dentatus articulator ARH.

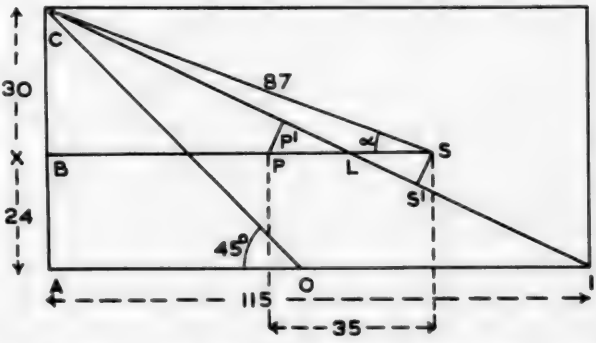

Fig. 3.—The Dentatus articulator ARH projected on the median sagittal plane. C is the center of the condyles. I is the contact point of the incisal pin and the incisal table. S is the contact point of the first incisors. S-P represents the plane of occlusion that is situated in the same level as the groove in the incisal pin of the articulator.

$$\text{Thus, } Cs = \sqrt{100^2 - 50^2} = 87 \text{ mm.}$$

The Balkwill angle α may be found in Δ CBS:

$$\sin \alpha = \frac{BC}{CS} = \frac{30}{87} = 0.3448 \text{ or } \alpha = 20^\circ\ 10'$$

This value for α is almost equal to those found by Köhler,[3] Hart,[4] and Bergström.[5]

We limited our studies of the movements to the paths of 2 points, P and S. S is the top of the Bonwill triangle, P was chosen on the straight line BS, at the distance of 35 mm. from S, in accordance with the investigation of Keith.[6] He stated that the average palatal length of Englishmen is 50 mm. Thirty-five millimeters is about the distance from I to the mesiobuccal cusp of the second molar, measured in the sagittal plane.

Gysi[7] stated that the inclination of the sagittal condyle path varies from 5° to 54°, with an average of 33°. This study was made especially to show the principal differences between the several types. As the choice of the angle is not of essential importance, an angle of 45° was chosen for our calculations.

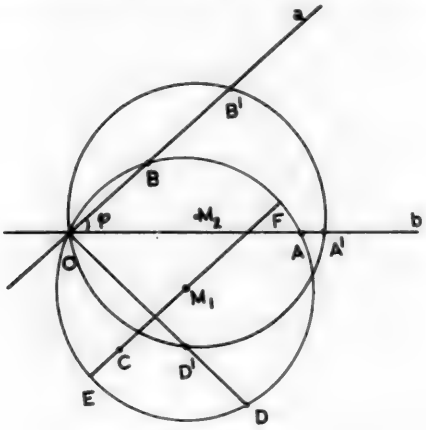

Fig. 4.

GENERAL CONSIDERATIONS CONCERNING THE MOVEMENTS OF THE TYPES I[a], II[a], III[a], AND IV[a]

Type I[a].—Two points A and B of the moving plane (Fig. 4) move along 2 intersecting straight lines of the fixed plane (if these straight lines were either parallel or identical, then each point of the moving plane would move in a straight line). a and b are the fixed straight lines, making an angle ø, and intersecting in O. The distance A-B is fixed (= A^1B^1). As ø is also fixed, the circumscribed circles of Δ ABO and Δ A^1B^1O have a radius of the same length r; O is a point on both circles. All possible positions of AB are to be found by constructing any circle with radius r passing through O.

What will the movement be of an arbitrary point D lying on the circle with M_1 as center during the movement of AB to A^1B^1? D moves together with AB. Draw a line through D and O. DO intersects circle M_2 at D^1. Because circle M_1 and M_2 have the same radius r, and because ∠ A^1OD^1 = ∠ AOD, it follows that arc, A^1D^1 = arc AD. This means that D^1 is the new position of D. Accordingly during the movement, any point of circle M_1 is describing a straight line through O, because D has been chosen arbitrarily.

How will the movement be of an arbitrary point C in the moving plane and not on the circle? Connect C with M_1. CM_1 intersects circle M_1 in two diametrical points E and F. During the movement E and F are moving along two straight lines e and f, which intersect in O (see above). As EF is the diameter of circle M_1, e and f are perpendicular (Fig. 5).

From Fig. 5 it can be deduced that, if x, y are the coordinates of C with regard to X and Y axes, $x = q \cos \theta$, and $y = p \sin \theta$. Thus, $\cos \theta = \frac{x}{q}$ and $\sin \theta = \frac{y}{p}$ and in combination with $\cos^2 \theta + \sin^2 \theta = 1$, this gives $\frac{x^2}{q^2} + \frac{y^2}{p^2} = 1$.

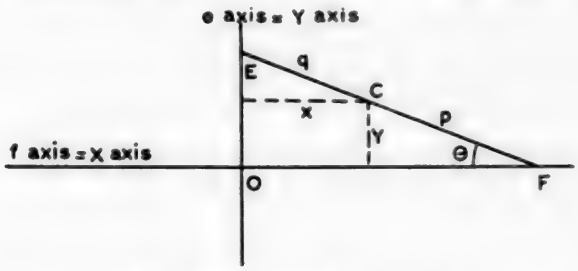

Fig. 5.—EC = q; FE = 2r; FC = p.

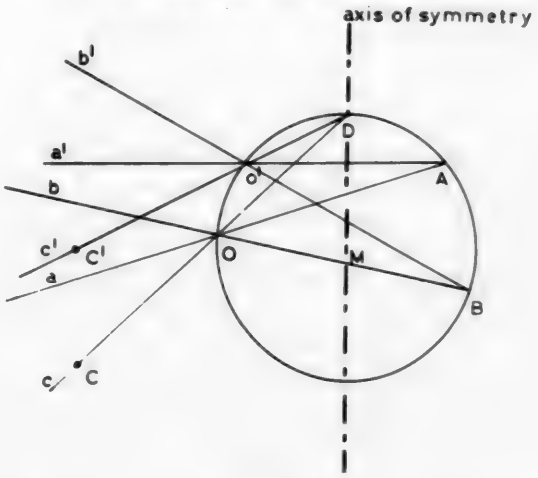

Fig. 6.

This means that the path of C is an ellipse with e and f as main axes (p and q are the lengths of the half axes of the ellipse). Only in the case that C is identical with A or B this ellipse is degenerated to a straight line.

Type II^a.—Two fixed points, A and B. Two lines, intersecting in O, of the moving plane are moving along A and B, respectively. Thus, the point O describes the circumscribed circle of Δ OAB. $\angle$ AOB is constant (see Fig. 6 and Fig. 1, II^a).

C may be any point of the moving plane. Connect C with O. The second intersecting point of CO (= c) with the circle is D. In a new position of Δ OAB line c′ = C′O′ is going necessarily through D, because ∠ COB = ∠ C′O′B. CO = C′O′ = g.

The locus of the points C is to be found in the following way: Construct the circumscribed circle of Δ OAB. Consider all straight lines c through D. These straight lines intersect the circle in points $O,O^1, \ldots$. The points C are to be found by circling round the distance g = OC with the points O being the center. The intersecting points of these circles with the straight lines c, are the points C, which were to be found. DM is the axis of symmetry of the locus.

When MD = m, the path of C shows a loop in D when g < 2m. The path shows a turning point when g = 2m, and the path consists of 2 loops when g > 2m. In the first case the path is called "limaçon." In the second case, the "limaçon" is called "cardioïde." In the latter case, D is an isolated point. When g < 2m, D is a double point.

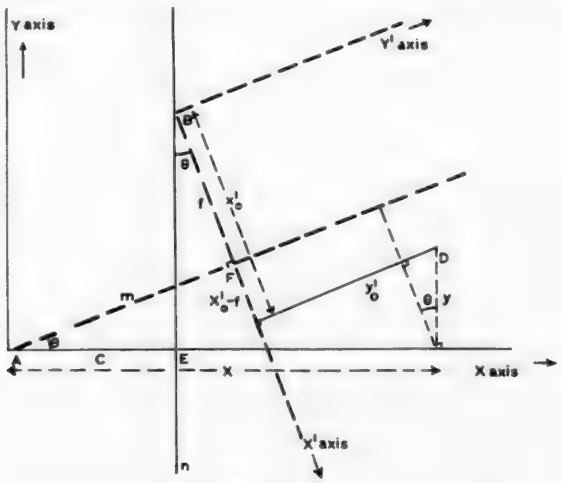

Fig. 7.

Type III[a].—One straight line m of the moving plane is going through a fixed point A of the fixed plane; a fixed point B of the moving plane moves along a straight line n of the fixed plane (Fig. 7).

The distance between A and n is c. m is the straight line moving through A. B is the point moving along n. The distance between B and m is f. The straight line AE is chosen as X axis (the direction from A to E is positive). The Y axis meets the X axis in A.

To simplify the calculation another coordinate system is chosen. The X′ axis is chosen as positive in the direction of BF. The Y′ axis is chosen perpendicular to the X′ axis in B. We will investigate how a certain point D, fixed to BFm, is moving together with BF. The coordinates of D in the X′Y′ system, are x_0 and y_0. In the X Y system they are x and y. From Fig. 7 is to be deduced that

$$x - c = x'_0 \sin\theta + y'_0 \cos\theta$$
$$x'_0 - f = x \sin\theta - y \cos\theta \text{ or:}$$
$$\sin\theta = \frac{y\,(x-c) + y'_0\,(x'_0 - f)}{y'_0\,x + x'_0\,y} \text{ and}$$
$$\cos\theta = \frac{x\,(x-c) - x'_0\,(x'_0 - f)}{y'_0\,x + x'_0\,y}\ .$$

The equation concerning the path of point D thus is, with $\sin^2\theta + \cos^2\theta = 1$:

$$(x-c)^2\,(x^2+y^2) - 2\,(x'_0 - f)\,(x-c)\,(x'_0 x - y'_0 y) - (y'_0 x + x'_0 y)^2 + (x'_0 - f)^2\,(x'_0{}^2 + y'_0{}^2) = 0.$$

This curve is one of the fourth order.

When $y \to \infty$, x in general $\to c + x'_0$ or $c - x'_0$.

This is to be deduced geometrically from Fig. 7. When c = f the movement is called stretchable. When c does not equal f the movement is not stretchable. The curve is then composed of two different parts, only one of which may function as the path. From a kinematical point of view the movement is then incomplete. Only by means of reflection do we arrive at the other branch.

When f = o, the movement is called the common conchoidal movement. In this case, the path of D is called a conchoide. When f does not equal o we speak of general conchoidal movement.

Type IV^a.—This type is to be considered essentially the same as type III^a, only the moving straight lines of type III^a are now points, while the points of III^a are now straight lines. As a matter of course, the path of D is not the same as that of III^a.

CONSTRUCTION OF THE PATHS OF S AND P IN THE TYPES I^a, II^a, III^a, AND IV^a

Point S: (Fig. 7.) The straight line QS is horizontal, c forms with i an angle of 45°. CI = 127 mm. for $CI^2 = CA^2 + AC^2 = 54^2 + 115^2 = 127^2$. OI = 61 mm.

In Fig. 8 the path of S is constructed in the types I^a and II^a.

Construction I^a.—M is the center of the circumscribed circle of Δ COI. SM intersects the circle in E and F. OE and OF then are the axes of the ellipse, which is to be described by S.

From the points E_1, E_2 and E_3 the distance EF is circled round.

Thus: $EF = E_1F_1 = E_2F_2 = E_3F_3 = EF_4 = E_1F_5 = E_2F_6 = E_3F_7$.

Points of the S path are to be found by circling round the distance FS from F_1, F_2 . . . . . F_6, F_7, respectively. In this way we find S_1, S_2 . . . . . S_6, S_7, respectively. The curve through these points is the path of S in type I^a.

Construction II^a.—OS is produced until it intersects the circle in D. We draw the straight lines: DF, DO_1, DO_2 . . . . . DO_6, DO_7, respectively. From the points F, O_1 . . . . . O_6, O_7, respectively, the distance OS is circled round. This gives the following points: S′, S'_1, S'_2 . . . . . S'_6, S'_7, respectively. The straight line DM is the axis of symmetry of the curve through these points and intersects the curve in S'_8.

The part of the curve we drew belongs to the smallest loop of the limaçon.

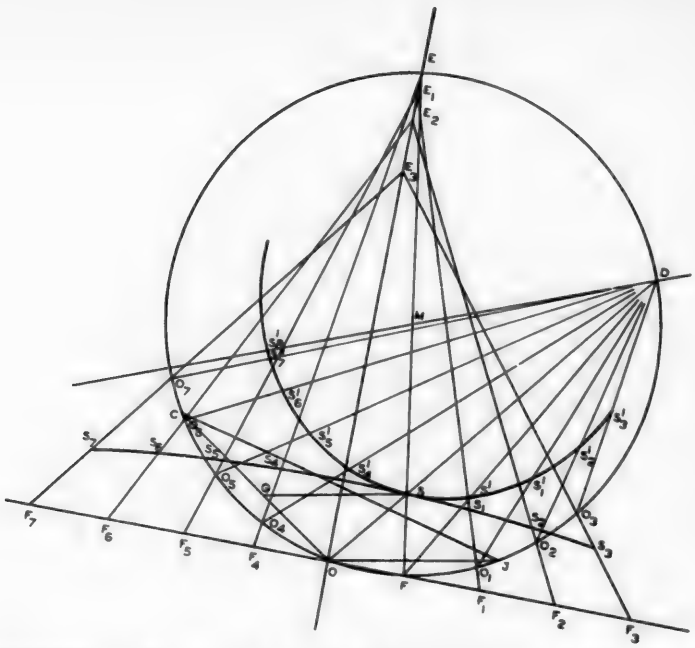

Fig. 8.—Construction of the path of S in the types I^a and II^a. S is the plane of occlusion parallel to OI.

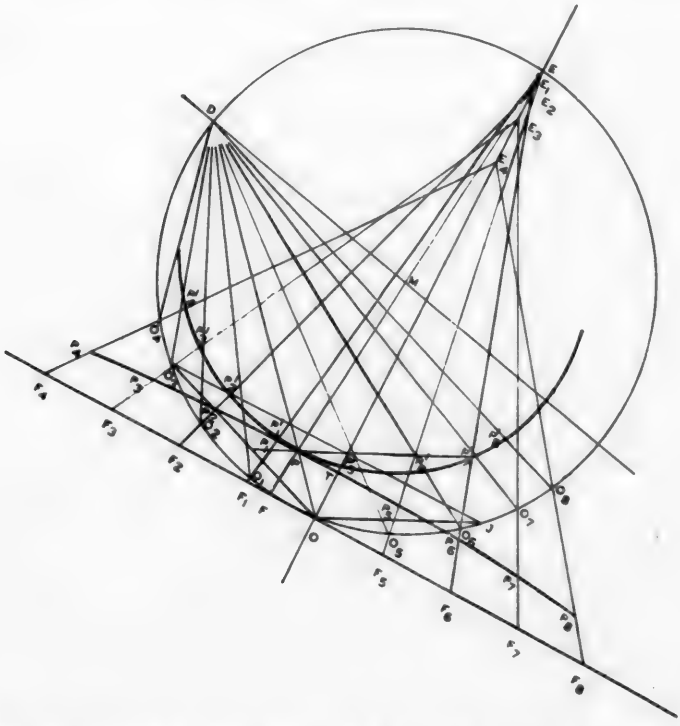

Fig. 9.—Construction of the path of P in the types I^a and II^a essentially. The construction is the same as in Fig. 7. In type I^a, P describes the curve through the points P_1—P_8. In type II^a, P describes the curve through the points P, P'_1—P'_8. Circle M is again the circumscribed circle of OI. O_3 is the point C from Fig. 7.

In Fig. 9, the path of the point P is constructed in the types I^a and II^a. OE and OF are the axes of the ellipse which P describes in type I^a. DM is axis of symmetry of the limaçon which P describes in type II^a.

Construction III^a (Fig. 10).—CO is moving through the point C. The point I is moving along OI. The distance IE remains constant. A new position is to be found by circling round the distance IE from a certain point I_1 on OI. The tangent from C to this circle is the new position of E. S moved together with IE. Thus the new position of S is to be found by circling round the distances ES and IE from E_1 and I_1, respectively. Thus, Δ EIS $\cong \Delta$ $E_1I_1S_1$.

In type III^a, the path of S is going through S_1 S, and S_2. In the same way, point S_2 is found. Each point of the path is to be constructed in this way.

Construction IV^a.—In this case the straight line OI is moving through I. C moves along the straight line OC.

Consider a new position C_1 of the point C.

We describe a circle with C_1 as center, and with the distance CD as radius. The tangent from I to this circle is the new position of the straight line IO. The intersection D_1 is the new position of D. S did move together with CD, thus Δ CDS$\cong \Delta$ $C_1D_1S'_1$.

The point S'_2 is found in the same way. Here Δ CDS $\cong \Delta$ $OD_2S'_2$; O is to be considered as the new position of C. In type IV^a the path is going through S'_1, S, S'_2. Each point of the path is to be found in this way.

In Fig. 11, the path of P is drawn in the types III^a and IV^a.

In the first case, I is moving along OI and CO moves through C. By continuous examination of Δ EIP we find the points of the P path. In type III^a the curve is P_1, P, P_2. In type IV^a, we continually consider the position of Δ CDP and in this way we find the P path, going through P_2', P, P_1'.

THE MOVEMENT OF THE HUMAN MANDIBLE

We now have to find the movement of P in the human mandible. It is not necessary to analyze the movement of S, because S describes a straight line.

Since the protrusive movement is to be considered as the movement of a line, two points of which are moving along two fixed straight lines, the path of any point is an ellipse (type I^a).

This had been proved by Bakker.[8] It will be clear that this ellipse is not the same as the one we usually find in any particular articulator. It is only the same ellipse when all lengths and angles are equal.

The preceding considerations show that the types I^a – IV^a for the points P and S, and also for any point, give essentially different paths. We have confined ourselves to the construction of the paths of P and S in a special case, in which the planes c and i meet in an angle of 45°, while SP is parallel to i. It is evident that this special choice is of no importance to the principal difference of the various types.

The analysis of the human mandible has shown that, if the paths of C and I are straight, the path of any point that is attached to the line moving over c and i is an ellipse, the form of which depends on the angle between c and i and the length of the moving line.

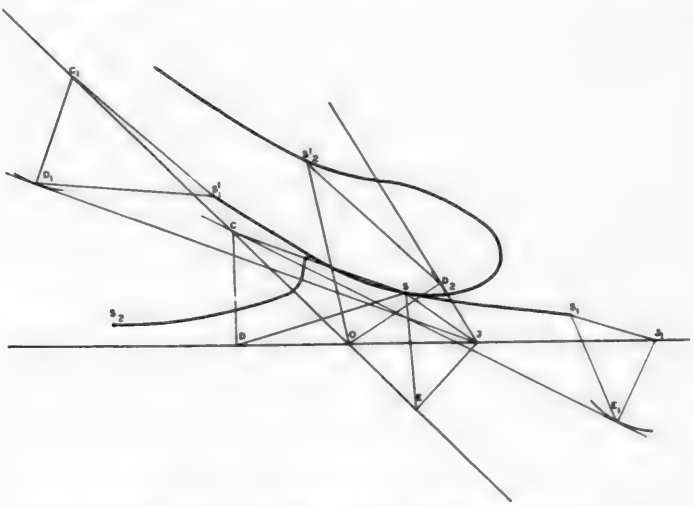

Fig. 10.—Construction of the path of S in the types III^a and IV^a.

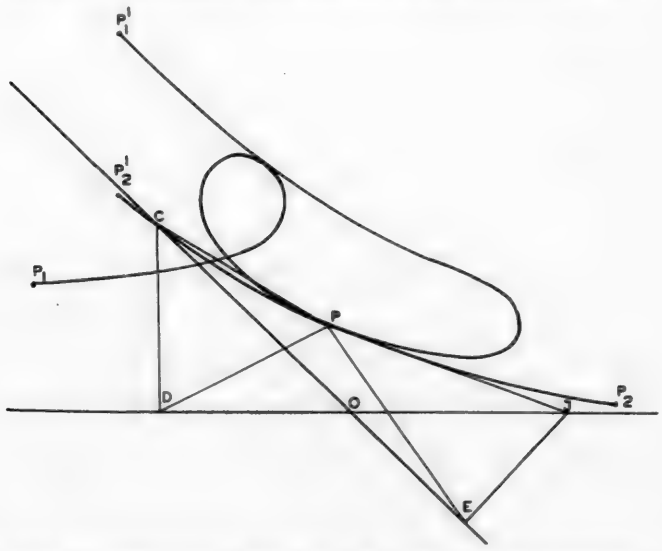

Fig. 11.—Construction of the path of P in the types III^a and IV^a.

Considering the results of the analysis of the articulator it appears that: Types I^a and I^b give an ellipse for the lower member and types II^a and II^b give a cardioid for the lower member (Figs. 8 and 9).

Types III^a, II^b, IV^a, and IV^b give a general chonchoidal movement (Figs. 10 and 11), independent of the choice of the angle between c and i and the length of the moving line.

SUMMARY AND CONCLUSION

1. Articulators with joints and an incisal pin can be divided into 8 groups (types I^a, I^b, II^a, II^b, III^a, III^b, IV^a, and IV^b).

2. The types indicated with *a* differ from those indicated by *b* in such a way that in the first-mentioned group, the lower member is movable, and in the second group the upper member. The relative position of any point attached to the lower member in respect to the upper member is important but it is of no importance whether the upper or the lower member is movable. The analysis of $I^a - IV^a$ is sufficient.

3. Type I^a (and consequently also I^b) results in an elliptical movement. In principle the movement is the same as that of the human lower jaw and only different in that the length of the moving line of the articulator is considerably greater than that of the human jaw, the angle between c and i may be chosen identically.

4. Type II^a (and consequently II^b) results in a cardioid movement, essentially different from that of the human jaw. The points of the path in which we are interested are, in general, situated in the small loop of the cardioid. The radius of the cardioid is short with regard to the radius of the ellipse.

5. Types III^a and IV^a (and consequently, III^b and IV^b) result in a general conchoidal movement. These curves also differ essentially from those of the human mandible.

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A STUDY OF THE FATIGUE CHARACTERISTICS OF SILVER AMALGAM

ELVIN G. WILKINSON, D.D.S., M.S., AND DONALD C. HAACK, M.S.

From the Department of Pedodontics, College of Dentistry, and the Department of Engineering Mechanics, College of Engineering, University of Nebraska, Lincoln, Neb.

INTRODUCTION

FACTORS affecting the quality and life of silver amalgam restorations can probably be placed in 3 major groups: (1) the dentist's skill in manipulation of the restorative material, (2) the shape of the cavity preparation, and (3) the strength of the material. The skill of the dentist is a subjective factor which has necessarily been eliminated from this investigation.

Shapes of cavity preparations which will enhance the physical properties of silver amalgam and result in the minimal stresses in the finished restoration have been established.[1, 2, 3]

The physical properties of silver amalgam have been thoroughly investigated by individuals and for companies manufacturing the various alloys. These investigations, however, have been limited to the static strength of silver amalgam, and the test conditions do not closely approximate those found in the mouth.[4, 5]

In the mouth, silver amalgam restorations are subjected to fluctuating stresses and, for this reason, the writers believe that the fatigue strength of the material is actually of greater importance than the static strength. According to Davis, Troxell, and Wiskocil,[6] "Most structural assemblages are subject to variation in applied loads, causing fluctuations in stresses in the parts. If the fluctuating stresses are of sufficient magnitude, even though the maximum applied stress may be considerably less than the static strength of the material, failure may occur when the stress is repeated a sufficient number of times. A failure induced in this manner is called a 'fatigue failure'." These authors[6] also stated, "It has been found that for most materials there is a limiting stress below which a load may be repeatedly applied an indefinite number of times without causing failure. This limiting stress is called the endurance limit. The magnitude of the endurance limit depends on the kind of stress variations to which the material is subjected."

The purpose of this investigation is to establish a method for the testing of silver amalgam under fluctuating stress conditions (fatigue) and to interpret

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This study represents a portion of the thesis submitted by the senior author in partial fulfillment for the degree of Master of Science in Dentistry in the Graduate College of the University of Nebraska.

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the data obtained. A review of the literature reveals that only brief mention is made of fatigue in one textbook on dental materials. No research appears to have been conducted to determine the fatigue strength of silver amalgam.

SELECTION OF EQUIPMENT

The tests were conducted in the Materials Testing Laboratory of the Department of Engineering Mechanics of the University of Nebraska. Of the fatigue testing and vibration equipment available, it was decided to use the Sonntag Universal Fatigue Testing Machine. This decision was based on the following factors: (1) The Sonntag machine is a standard piece of equipment readily available in other laboratories. (2) It can be adjusted to produce a fluctuating stress condition for which the stress in the compressive phase of the cycle is not equal to the stress in the tensile phase of the cycle. (3) It can be readily adapted to the use of relatively small specimens. (4) Specimens for use in the Sonntag machine require no threading or other complicated machine operations which could not be easily performed on silver amalgam.

Fig. 1 shows a drawing of the internal workings of the testing machine. The synchronous motor (A) rotating at 1,800 r.p.m. rotates the unbalanced weight (*B*). The rotation of this unbalanced weight causes a vibration in much the same way that a vibration is caused by an unbalanced wheel of an automobile. The amount of unbalance of the weight can be varied by changing the radius of the center of gravity of the weight relative to the axis of rotation. The vertical component of the centrifugal force causing the vibration is transferred through the platen and lower collet (*C*) to the specimen (*D*). The reaction to this force is supplied by the upper collet (*E*), which is rigidly attached to the large spring supported mass (*F*). This causes the specimen to be subjected to a sinusoidal stress variation with a frequency of 30 cycles per second. In addition, a static stress may be imposed on the fluctuating stress in the specimen. This is done by holding the calibrated springs (*G*) in a deformed position by turning the screw (*H*). The amount of force exerted by these calibrated springs can be determined by reading the dial indicator (*J*). While the machine is running, the total number of cycles is registered on a counter and, when the specimen breaks, the limit switches (*K*) shut off both the machine and counter. With both the fluctuating and static loads causing stress in the specimen, the variation of stress with time is of the form shown in Fig. 3.

PROCEDURE FOR MAKING SPECIMENS

A specimen of silver amalgam, $\frac{3}{16}$ in. in diameter and about $\frac{7}{8}$ in. in length, is the smallest that is practicable for testing in the Sonntag apparatus.

The mold in which the amalgam specimens were made is a simple cylindrical cavity of $\frac{3}{16}$ in. diameter in a block of clear acrylic with a detachable base. The top of the cylindrical cavity was reamed somewhat larger to permit easier placement of the amalgam into the mold. Fig. 2 shows this mold at the left.

About 4 Gm. of silver alloy and 6.4 Gm. of mercury were needed to make a specimen of sufficient size to be used in the Sonntag machine. The alloy and mercury were dispensed from ordinary dental alloy and mercury dispensers and placed in gelatin capsules. Each capsule of alloy weighed about 1 Gm. and each capsule of mercury weighed about 1.6 Gm. Thus, a ratio

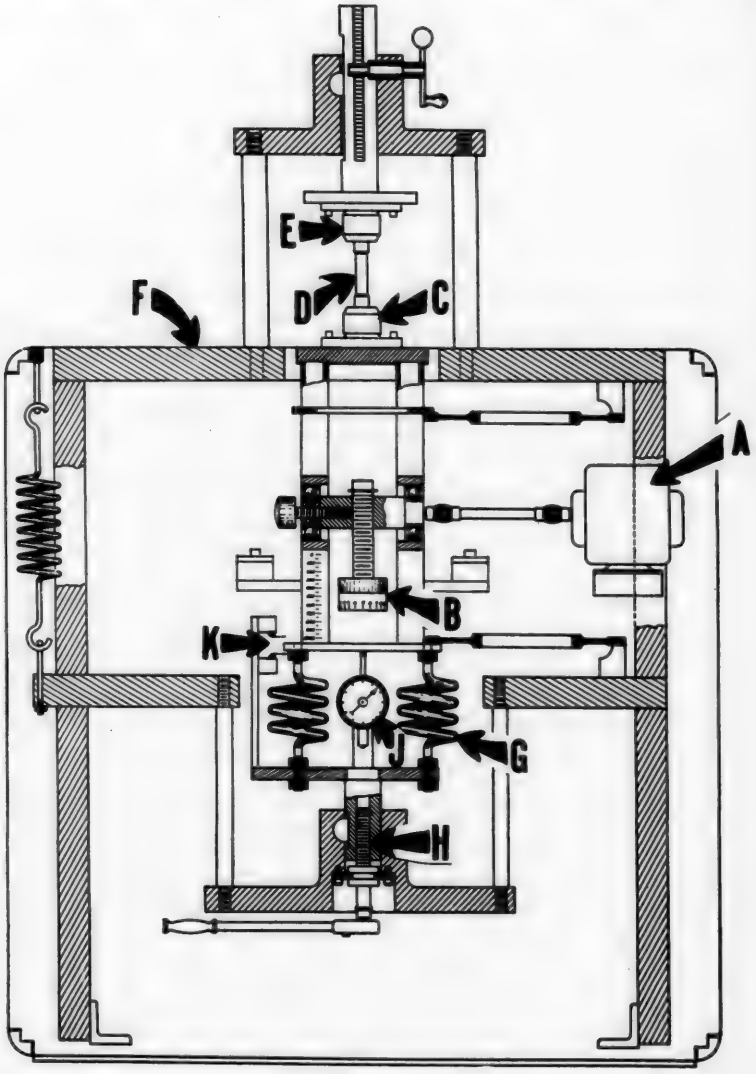

Fig. 1.

of 8 parts mercury to 5 parts alloy was maintained. Each capsule was carefully weighed to the nearest hundredth of a gram and the weight recorded on a data sheet. By subtracting the weight of the alloy from the weight of the

me ury with which it was to be combined, the weight of the mercury which was to be removed to obtain a 5:5 ratio of alloy to mercury was established.

The first mix was made by using 1 capsule of alloy and 1 of mercury. The materials were placed in a Bakelite capsule containing a pestle and triturated for 10 seconds on a mechanical triturator (Wig-l-bug). The amalgam was then placed in a squeeze cloth and the excess mercury was expressed into the pan of a balance which had already been adjusted to the amount of mercury necessary to be expressed from the particular mix in order to obtain a 5:5 ratio of alloy to mercury. This resulted in a dry but workable mixture which was crumbled and placed into the mold. The amalgam was then tamped into place with a flat-ended punch and a light hammer before the next mix was inserted. The same procedure was followed using the other capsules of alloy and mercury.

Fig. 2.

To condense the alloy thoroughly, the mold was placed in a hydraulic press in which a $\frac{3}{16}$ in. diameter metal cylinder had been mounted. The cylinder was fitted into the end of the mold and intermittent pressure was applied ranging from 11,000 to 29,000 pounds per square inch. This process was continued until the amalgam was thoroughly condensed (2 to 3 min.). The entire procedure took about 12 minutes. The mold was then removed from the press and allowed to stand for 24 hours. The base of the mold was then removed and pressure was applied with the press in order to push out the specimen. All specimens were tested at a uniform age of 7 days. This permitted the specimen to age and also allowed time for grinding and polishing.

The weight (Gm.) and volume (c.c.) of each specimen were determined and from these data the specific gravity of each silver amalgam specimen was calculated. This information was used as a control of uniformity of the silver amalgam. The specific gravity of all specimens was about 11.6.

Specimens used for fatigue testing are usually cylinders with a reduced central section. This shape is preferred because it causes the failure to occur in the central section of the specimen which is free from unknown stresses, such as those caused by the collets which hold the specimen in the fatigue testing machine. During the interval between manufacture and testing, the

specimen was placed in the chuck of a metal lathe on which a grinding machine had been mounted. The specimen was rotated slowly and the rapidly rotating grinding wheel was brought into contact with it. The central portion of the specimen was reduced to a minimum diameter of about .113 in. and the ends to about .187 in. Compressed air was blown on the specimens in order to prevent heating. Fig. 2 shows the specimen at the right.

Davis, Troxell, and Wiskocil[6] stated that "The surface finish of a test specimen has a definite effect on the fatigue strength: the rougher the finish the lower the endurance limit. In fact, a very rough surface finish, particularly if the scratches or machine marks are normal to the applied force, may lower the endurance limit of a metal by as much as 15-20 per cent in comparison with the endurance limit for a polished surface. However, a part which is machine polished may fail more rapidly than one with a good tool finish, because of the tension set up in the skin by the heat of the polishing."

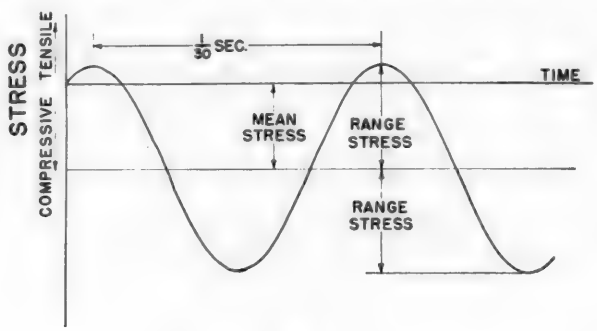

Fig. 3.

Therefore, to eliminate irregularities, all specimens were polished highly, using the following procedure: An electric drill was placed in a vise. The specimen was placed in the chuck of the drill and rotated. A rag wheel on a mandrel in a dental handpiece was used with a paste of flour pumice to remove all visible scratches from the surface of the specimen. Care was taken to keep the specimen cool during the polishing process.

Since the maximum compressive strength of silver amalgam is about 10 times its maximum tensile strength, the compressive stress used in the fatigue testing was arbitrarily made 10 times the tensile stress.[5]

The minimum cross-sectional area of each specimen was calculated,

$$A = \frac{\pi D^2}{4}.$$

The selected values of tensile and compressive stress were used to determine the mean and range stress for each specimen. In this calculation, compressive stresses were treated as negative values and tensile stresses as positive. The mean stress is then $(S_t + S_c)/2$, and the range stress is $(S_t - S_c)/2$. The significance of range and mean stress and the variation of stress with time is shown graphically in Fig. 3. Adjustment of the machine required

that the mean and range stresses be converted to forces. This conversion was accomplished by multiplying the mean and range stresses by the cross-sectional area.

In order to avoid any bending stress in the specimen, great care had to be taken in clamping the specimen in the collets of the Sonntag machine.

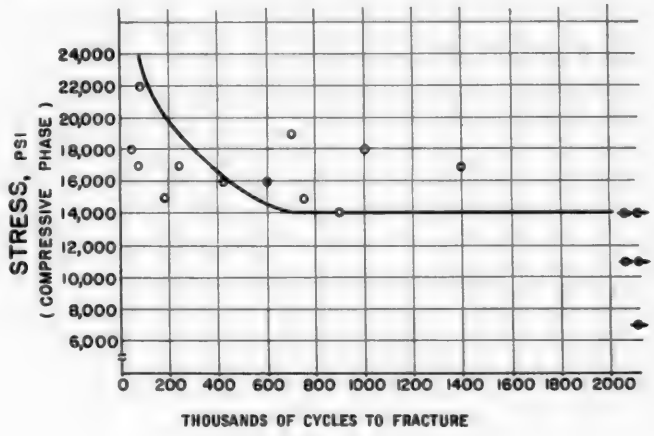

Fig. 4.

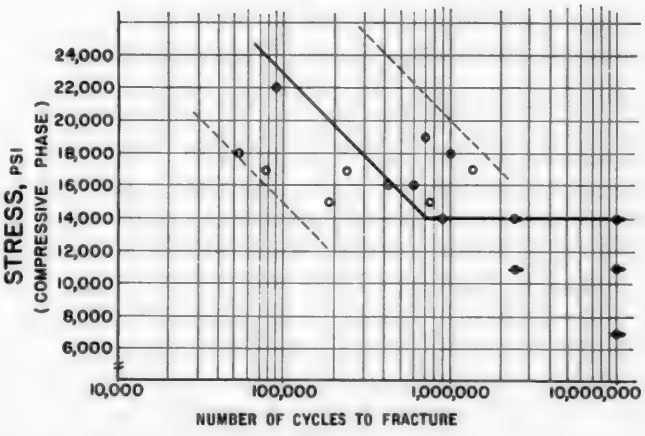

Fig. 5.

When the specimen broke, the number of cycles was read to the nearest thousand, from the counter. Any specimen enduring over 10,000,000 cycles was considered to be stressed below the endurance limit.

DATA ANALYSIS AND DETERMINATION OF ENDURANCE LIMIT

In order to determine the endurance limit of the material tested and to draw other conclusions from a series of fatigue tests, certain analytical procedures are required. The following procedures were used in this investigation.

In plotting the data obtained from the fatigue tests, the stress for each test was plotted as an ordinate against the number of cycles required to produce failure in the specimen as an abscissa. Only the compressive stress has been plotted, but the reader is reminded that this compressive stress was alternated with a tensile stress one-tenth as great. Fig. 4 shows the results plotted in rectangular coordinates, whereas Fig. 5 shows the same results in semilogarithmic coordinates. The points with the small horizontal arrow represent specimens that did not break during the test. Such points are plotted at the number of cycles at which that particular test was abandoned, but they must be interpreted as representing an infinite number of cycles. The endurance limit is the stress (in this case, that of the compressive phase of the fluctuation) at which the curve of Fig. 4 flattens out and becomes horizontal. The same thing may be found at the point at which the straight line of Fig. 5 breaks sharply and becomes horizontal. All tests were made using a single commercially available, American Dental Association approved, alloy. The endurance limit of this alloy, as determined from these tests, is 14,000 psi.

Fatigue data are always subject to considerable scatter, and fatigue tests of silver amalgam are certainly no exception. These data show somewhat greater scatter than most fatigue tests, and this fact may result from variation among the specimens despite carefully controlled conditions of fabrication. The location of the straight line of Fig. 5 was determined by statistical procedures so that it represents the regression line of X on Y. Such a line minimizes the sum of the squares of the horizontal distances from the line to the individual data points.[7] Statistical methods were used to determine the area of 90 per cent probability as bounded by the parallel dotted lines also shown on Fig. 5.[7] The slope and location of the regression line is not of great significance in determining the endurance limit. The endurance limit of the material tested is quite definitely determined at 14,000 psi, because all specimens failed at stresses above this value whereas none failed at stresses below 14,000 psi. The location of the regression line, however, would be valuable information for the interpretation of data from repeated blow tests on silver amalgam restorations of different shapes.

CONCLUSIONS

Suitable technics for the production of specimens for use in the fatigue testing of silver amalgam were developed. The production of adequately sound and homogeneous specimens was one of the principal stumbling blocks.

This study led to the development of a fatigue test for silver amalgam. The results show that, while a restoration might withstand static stress up to 63,000 psi or infrequent short duration stress applications of the same intensity, the highest fluctuating stress which this silver amalgam might be expected to withstand is only 14,000 psi, or 22 per cent of the maximum crushing strength.

The shape of the curve relating stress to the number of cycles causing failure has been determined. This is a secondary result which, although it has

little value relative to the use of silver amalgam as a material for dental restorations, would be of great value in the quantitative analysis of data obtained from tests designed to determine the relation of cavity form to the failure of silver amalgam when subjected to repeated blows.

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THE CONTAMINATION OF ANESTHETIC NEEDLES

DONALD T. BENNETT, F.D.S.R.C.S. (ENG.)

IN DENTISTRY, syringes and needles used for administering local anesthetics are commonly sterilized by boiling water.[1] Following sterilization, they may either be used at once, or maintained in a sterile condition by immersion in an antiseptic fluid or by other technics. Frequently, however, especially after an initial injection, syringes are left in the surgery with the needle exposed to the atmosphere. Sometimes the needle may be covered with a clean absorbent cotton roll.

A preliminary investigation was carried out to examine whether or not anesthetic needles became contaminated when exposed to the atmosphere or when covered with an absorbent cotton roll. The results reported are of a preliminary survey and are not intended to be conclusive.

EXPERIMENTAL PROCEDURES

In the experiments, dental cartridge syringes and 1 5/8 inch needles (minus the screw-on hub attachments) were sterilized by immersion in boiling water for at least 10 minutes. After sterilization the needles and syringes were placed aseptically within the cover of a sterile towel, where they were allowed to cool and dry.

The experiments were divided into two groups: In Group A, the needles and syringes were removed, using an aseptic technic, from the sterile towels. The working 1 inch of the dried needle was now dipped into sterile plasma, following which the needle was inserted into the normal position in the syringe. The assembled unit was placed on the bracket table in the surgery, with the needle exposed to the atmosphere. In Group B, the needles and syringes were prepared as in Group A, except that after being dipped in sterile plasma the needle was covered by being pushed into a "clean" absorbent cotton roll.

All the experiments were carried out in the busy clinic of a dental school. To provide maximum chances of aerobic contaminants settling on the needles, each needle was dipped into sterile plasma, following its removal from the cover of the sterile towel. By this means, everyday clinical conditions were simulated, as following removal from the sterilizer, antiseptic fluid, or from the oral cavity, the needle surface is invariably moist.

In both groups, the assembled units, with the needle uncovered or covered, were left exposed to the atmosphere for a period of 15 minutes. After this

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interval, the needles were removed from the syringes. In Group B, the absorbent cotton rolls were also removed. The needles were then immediately dropped into sterile test tubes containing sloppy agar.

All the agar tubes and the contained needles were incubated at 37° C. for 48 hours, and then examined for bacterial growth. If a negative result was obtained at this examination, incubation was continued up to 96 hours when, if still negative, the experiment was discontinued.

If positive, the agar growths were then subcultured both aerobically and anaerobically in blood agar plates for 12 hours at 37° C. Gram-stained microscopic smear preparations were made of all positive plates and the organisms identified.

RESULTS

No pathogenic organisms were detected in either Group.

In Group A, 5 positive cultures were obtained from the 50 needles examined. The positive cultures consisted of 2 gram-positive growths of diphtheroid bacilli, and 3 gram-positive growths of *Staphylococcus saprophyticus.*

In Group B, only 2 positive cultures were detected from 50 experiments. Both consisted of pure growths of gram-positive *Staphylococcus saprophyticus.*

CONCLUSIONS

Although the number of needles tested in this preliminary series is too small to permit definite conclusions to be drawn, it does prove that, under the experimental conditions employed, anesthetic needles may be contaminated by air-borne organisms, when exposed to the atmosphere.

The possibility of contamination by pathogenic organisms, even though this did not occur in the present series, must always be borne in mind. Thus, in general practice it would appear wise to use the syringe directly following its removal from the sterilizer, and not to leave the syringe on the bracket table prior to a second injection; alternatively, to maintain the syringe and attached needle in an antiseptic fluid or under aseptic conditions.

The covering of an exposed needle with a "clean" absorbent cotton roll appears to minimize the chances of air-borne contamination.

SUMMARY

A series of 100 sterilized local anesthetic needles were examined for possible bacteriologic contamination which might occur when such needles were exposed to the atmosphere, in circumstances reasonably similar to those which could arise in general practice.

The series was divided into 2 experimental groups. Group A, consisted of 50 needles which were freely exposed to the atmosphere for 15 minutes. After this interval, 5 needles (10 per cent), were found to be contaminated by saprophytic organisms. In Group B, a similar number of needles were exposed for a similar interval, but in this group the needles were covered with a "clean" absorbent cotton roll. In this group, only 2 needles (4 per cent) were contaminated with similar organisms. No pathogenic organisms were detected in either group.

The author expresses his gratitude to Dr. H. B. May, The London Hospital, for helpful discussions and for allowing him the facilities of his laboratory, to Mr. A. M. Horsnell, The London Hospital Dental School, for encouraging this study and for advice in the preparation of this communication, and to the Director of Dental Services, Royal Air Force, for permission to publish this paper.

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VISUALIZATION OF C^{14} IN THE TOOTH MATRIX AFTER ADMINISTRATION OF LABELED HEXOSES

Y. KUMAMOTO AND C. P. LEBLOND

Department of Anatomy, McGill University, Montreal, Canada

CARBOHYDRATES of high molecular weight have been detected in the matrix of dentin[1-6] and enamel,[4, 7, 8] but no information is available regarding the mode and site of their production. Recent reports have shown that labeled glucose is a precursor of certain carbohydrates, particularly hyaluronic acid and chondroitin sulfuric acid.[9, 10, 11] Glucose may also be a precursor of the carbohydrates appearing in dentin and enamel. Therefore, a radioautographic study of the tooth after administration of labeled glucose might be a source of data on the formation of carbohydrates in this structure. Accordingly, glucose labeled with C^{14} was injected into young rats and at various time intervals thereafter, their teeth were examined radioautographically.

The results indicated that, in the case of enamel, glucose may be a precursor of the carbohydrates of the matrix but, in the case of dentin, the labeled carbon of glucose is taken up in a component of the matrix which seems to be the protein, collagen. An interesting conclusion is the remarkable stability of this labeled component of dentin.

MATERIALS AND METHODS

In all experiments, 3-day-old rats were given labeled hexoses obtained from the Radiochemical Centre, Amersham, England. After a period varying from 3 hours to several months after administration, the animals were sacrificed by chloroform or ether. Upper and lower jaws were excised and fixed in a solution of 3 parts of 95 per cent alcohol to 1 part of commercial formalin neutralized with potassium hydroxide. Tissues removed 14 days or more after administration were decalcified in sequestrene (ethylene-diaminetetrasodium acetate). Histologic sections, either unstained or stained with hematoxylin-eosin or the periodic acid-Schiff (PA-Schiff) technic, were coated with Kodak NTB3 emulsion, dried and exposed in the dark. After a period of exposure varying from 3.5 to 12 months, the sections were processed through developer and fixer, dehydrated in alcohol and xylol, and mounted in Canada balsam or Permount.

Radioglucose uniformly labeled with C^{14} (50 μc per mg.) was used in 3 series of experiments at a dose of 30 μc per animal. In the first series, 2 animals received an injection by the intragastric, and 2 by the subcutaneous

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route; one animal of each pair was sacrificed at 3 hours and the other at 24 hours after the injection. In the second series, 3 animals were injected subcutaneously and sacrificed at 3, 24, and 72 hours, respectively. In the third series, 18 animals were injected intragastrically and 2 of them were sacrificed at each of the following times: 3 hours; 1, 3, 6, and 14 days; 1, 2, 4, and 6 months.

A parallel experiment was carried out with *radiomannose* (0.01 μc per mg.). Three animals received 23 μc of mannose-C^{14} subcutaneously and were sacrificed at 3, 24, and 72 hours later, respectively.

RESULTS

Whether radioglucose was administered intragastrically or subcutaneously, the distribution of C^{14} in the teeth was identical; and, therefore, the following description of the radioautographic reactions applies to both routes of administration. The radioautographs reproduced in Figs. 1-3 are not stained, so that every visible detail is due to a blackening of the emulsion in response to the rays emanating from C^{14}-labeled structures, present in the underlying section. In contrast, the radioautographs in Figs. 4-18 are stained and, thus, show not only the emulsion blackening due to radiocarbon, but also the stained tissues in the section.

The developing molar tooth examined at the 3-hour interval (Fig. 1) showed 2 dark lines of reaction. One of them, extending along the whole inner surface of the 3 cusps, was associated with the dentinal organ (Fig. 1, *D*). The other, an outer line, which appeared discontinuous and was best seen on the anterior and occlusal portions of the 3 cusps, was associated with the enamel organ (Fig. 1, *E*). The incisor tooth exhibited 2 comparable reaction bands, a circumpulpal one in relation to the dentin and another on the convex surface where enamel develops.

Examination of the molar and incisor teeth at a higher magnification revealed that the inner band of reaction emanated from predentin and could be assigned to that portion of predentin adjacent to the odontoblasts lining the pulp cavity (Fig. 10, arrow slanting upward). The outer band of reaction was limited to the portion of the enamel organ known as pre-enamel, that is, the area approximating the taller ameloblasts (Fig. 10, arrow slanting downward).

At the second interval, 24 hours after administration, both reaction bands were still clearly distinguishable around the first molar (Fig. 2) and incisor teeth. The inner band was increased in intensity (Fig. 11) but the outer band was fading, a fact which was particularly clear in the case of the incisor. The site of the inner band was no longer predentin, but the innermost portion of the dentin proper. The outer band, now absent from pre-enamel, appeared over the middle region of the enamel organ. It was less precisely limited than at the first interval (Fig. 11).

At the 3-day interval, the inner band which persisted without detectable change in intensity was seen to occupy the mid-portion of dentin, although remaining at the same distance from the dentinoenamel junction as at the

Figs. 1-3.—Unstained coated radioautograph of undecalcified developing upper first molar tooth of rats given C^{14}-glucose at 3 days of age. Exposure was for 3.5 months. The first or anterior cusp is on left-hand side. Anterior edge of the second molar is visible on right-hand side. (Mag. ×19.) Fig. 1, 3-hour interval. Fig. 2, 1-day interval. Fig. 3, 3-day interval.

A band form of radiocarbon deposition appears in pre-enamel (Fig. 1, *E*) and pre-dentin (Fig. 1, *D*) in the 3 cusps of the first molar. While the enamel band decreases in intensity (Figs. 2 and 3 *E*), the dentinal band shows no apparent decrease with time. Note reactions in ameloblasts, particularly the younger ones (Fig. 1, *YA*), pulp (Fig. 2, *P*), young odontoblasts (Fig. 1, *YO*), oral epithelium (Figs. 1 and 2 *OE*), alveolar bone (Figs. 1 and 3, *B*), cartilage (Fig. 1, *C*), nasal mucous glands (Fig. 1, *MU*).

previous interval (Figs. 3 and 12), whereas the enamel band became broader and fuzzier (Fig. 3, *E*) and, under high magnification, was represented only by scattered silver grains (Fig. 12).

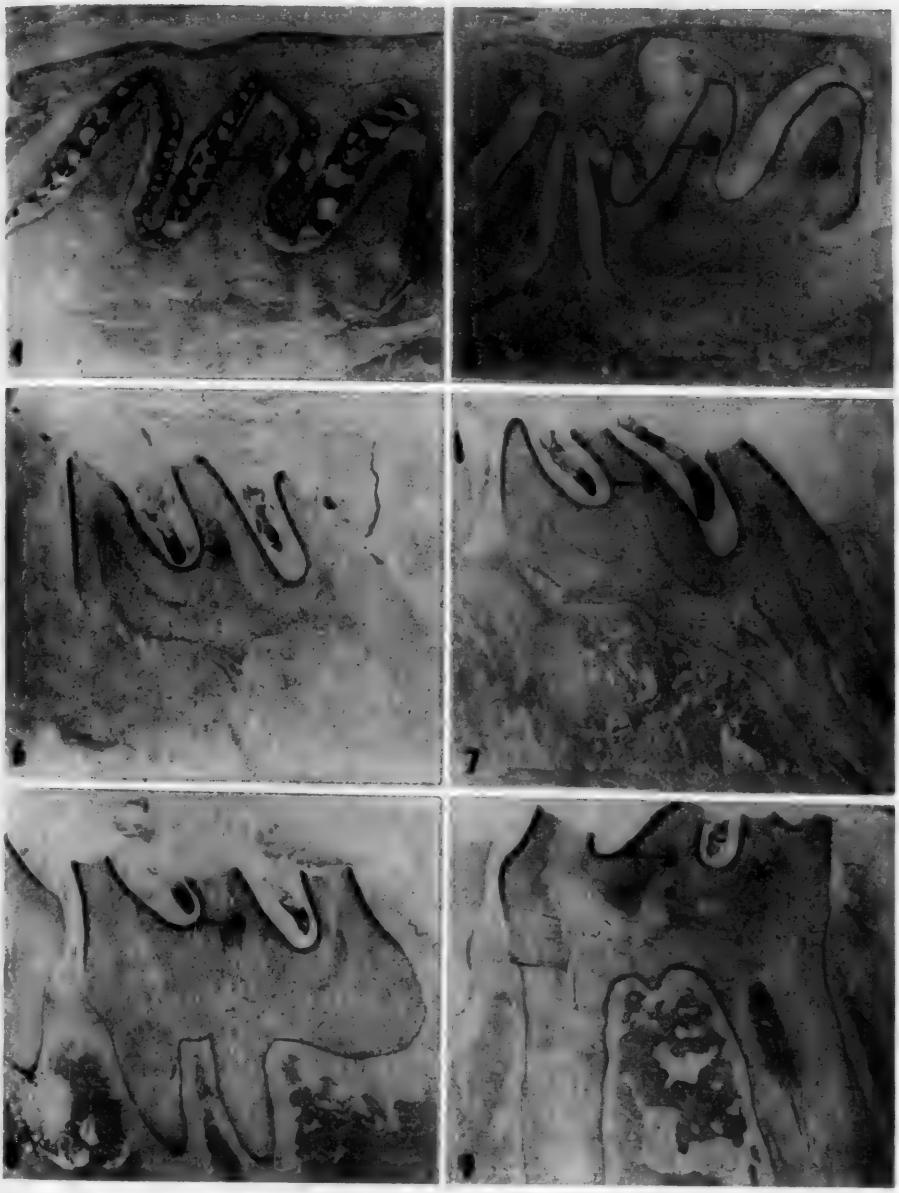
At the 6-day interval (Figs. 4 and 13), no definite enamel band was visible, although a few scattered grains were present, while, at 14 days and later, it was not possible to observe the enamel, since the latter had been eliminated by decalcification. At the 6- and 14-day intervals (Figs. 13 and 14), the dentinal reaction did not change in intensity, nor in its distance from the dentinoenamel junction; but its distance from the pulp increased gradually. This observation indicates that new layers of unlabeled dentin are continuously accumulated on the inside.

By the fourteenth day, the molar teeth had not yet erupted, so their occlusal surface was intact (Figs. 5 and 14). However, immediately after their eruption into the oral cavity, the surface became worn away by attrition. Accordingly, during subsequent intervals (1-6 months) the height of the crown decreased progressively (Figs. 6-9). The attrition of the tip of the cusps resulted in the loss of the portion of the reaction band present at the apex of the cusps (Figs. 6 and 15). The distance from the band to the dentinoenamel junction which could still be measured (at the top of picture in Fig. 15) was found to be approximately the same as at the 14-day interval (Fig. 14) and earlier. Meanwhile, the dentin had been continuously increasing in thickness as the odontoblasts receded and, as a result, the initial pulp cavity of the cusps gradually decreased in size (compare Figs. 2, 4, 5, and 6) and eventually was nearly obliterated (Fig. 7). The pulp cavity thus seemed to withdraw gradually to the base of the cusp, as shown by the increase in the distance from the occlusal surface (compare Figs. 14 and 15). With the passage of time, as the cusps were gradually worn away by erosion (Figs. 7-9), the dentinal reaction was still seen in their lower portion (Figs. 16-18).

Since the radioautographs obtained at the 1, 2, 4, and 6 month intervals had been exposed for the same duration and developed at the same time, they could be compared to one another. Such comparison showed that the intensity of the band reaction did not change with time (Figs. 15-18); neither did its relation to the dentinoenamel junction appear to be modified.

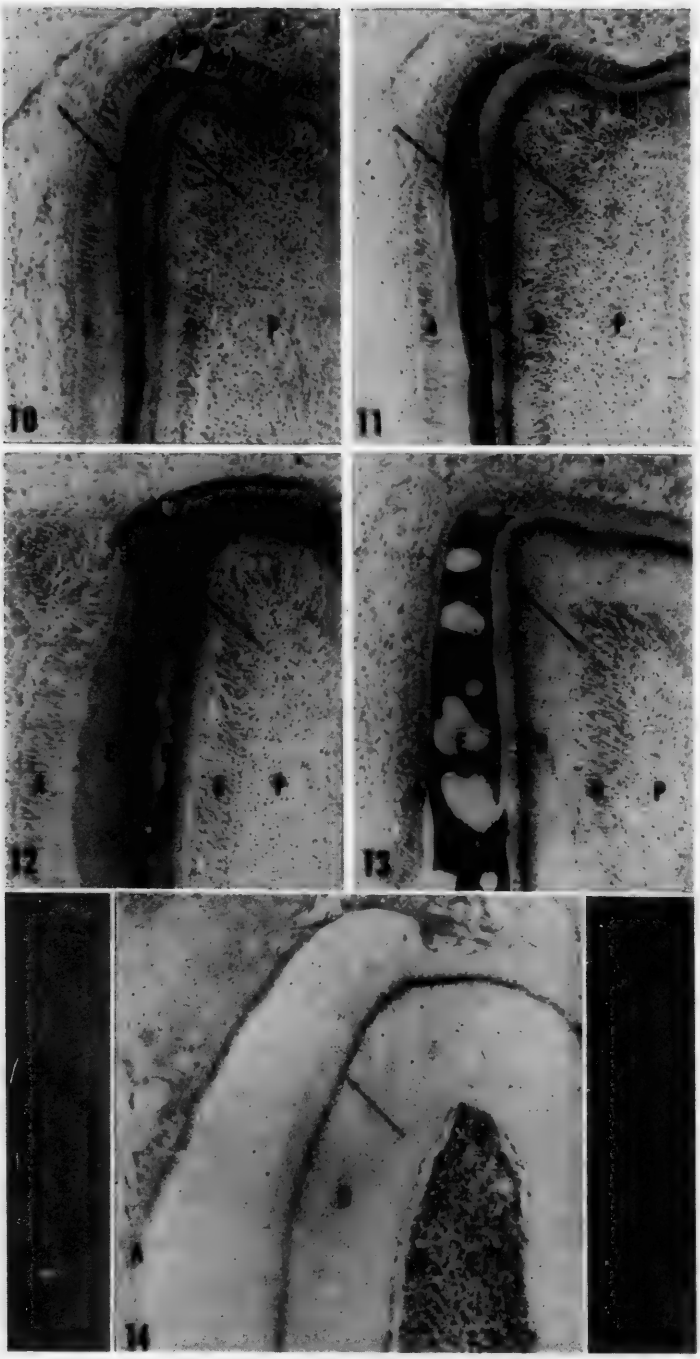
The results obtained after administration of *radiomannose* were, in all respects, similar to those obtained with glucose. Thus, under low power, the pictures showed the persistence with time of the dentinal band, and the rapid fading of the enamel band reaction (Figs. 19-21). At the higher magnification the intensity of the dentinal band was seen to increase until the 24-hour interval (Figs. 22 and 23) without change thereafter (Fig. 24). In contrast, the enamel reaction was maximal at 3 hours (Fig. 22) and later became more diffuse and less intense (Figs. 23 and 24).

Examination of tissues other than dentin and enamel revealed a widespread but similar distribution of radioactivity after radioglucose or radiomannose administration. Of the tissues associated with the tooth, the stellate reticulum (Fig. 19, left hand side), both of which decreased with time (Fig. 21). The

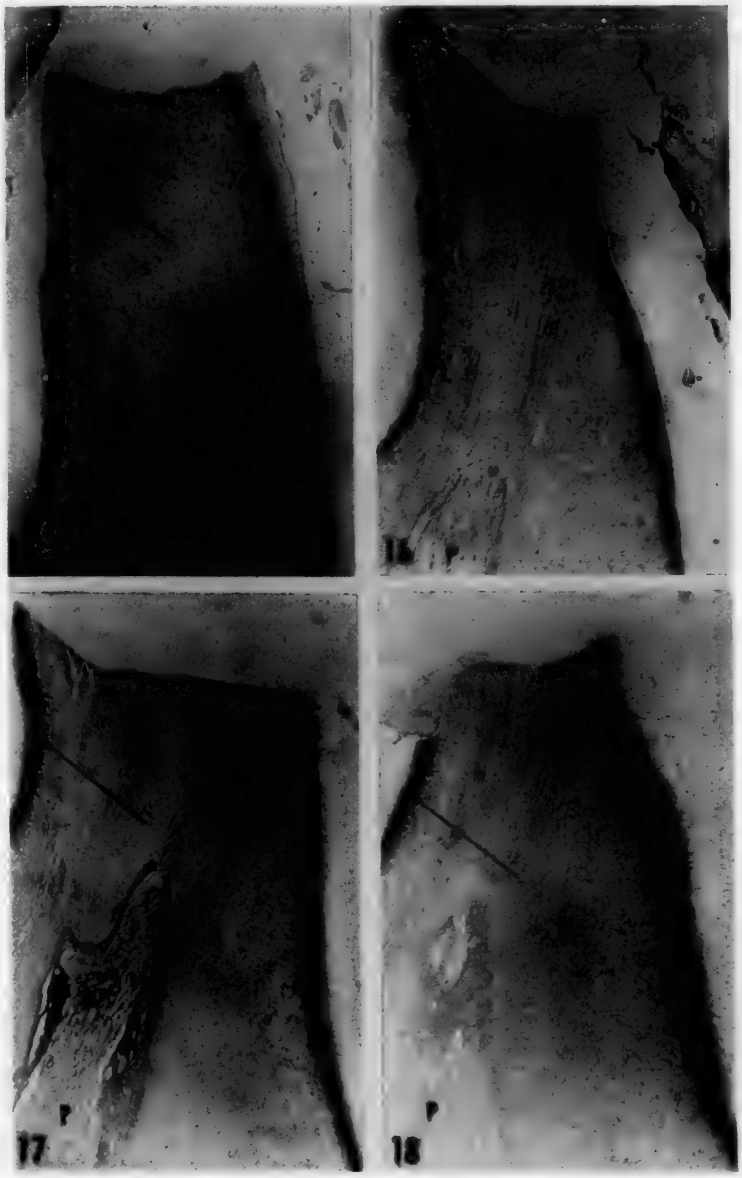


Figs. 4-9.—Stained coated radioautographs of molar teeth of rats given C^{14}-glucose at 3 days of age. (Mag. ×17.) Fig. 4, 6-day interval. First upper molar undecalcified, H and E stained, exposed for 3.5 months. Fig. 5, 14-day interval. First lower molar (second molar on the left), decalcified, H and E stained, exposed for 3.5 months. Fig. 6, 1-month interval. First upper molar (second molar on the left), decalcified, PA-Schiff stained, exposed for 7 months.. Fig. 7, 2-month interval (details as in Fig. 6). Fig. 8, 4-month interval (details as in Fig. 6). Fig. 9, 6-month interval. First lower molar (second molar on the left), decalcified, PA-Schiff stained, exposed for 7 months.

An intense dentinal reaction (*D*) persists at all time intervals without apparent change in pattern or intensity. As time goes on, the tips of the 3 cusps are steadily worn by attrition.

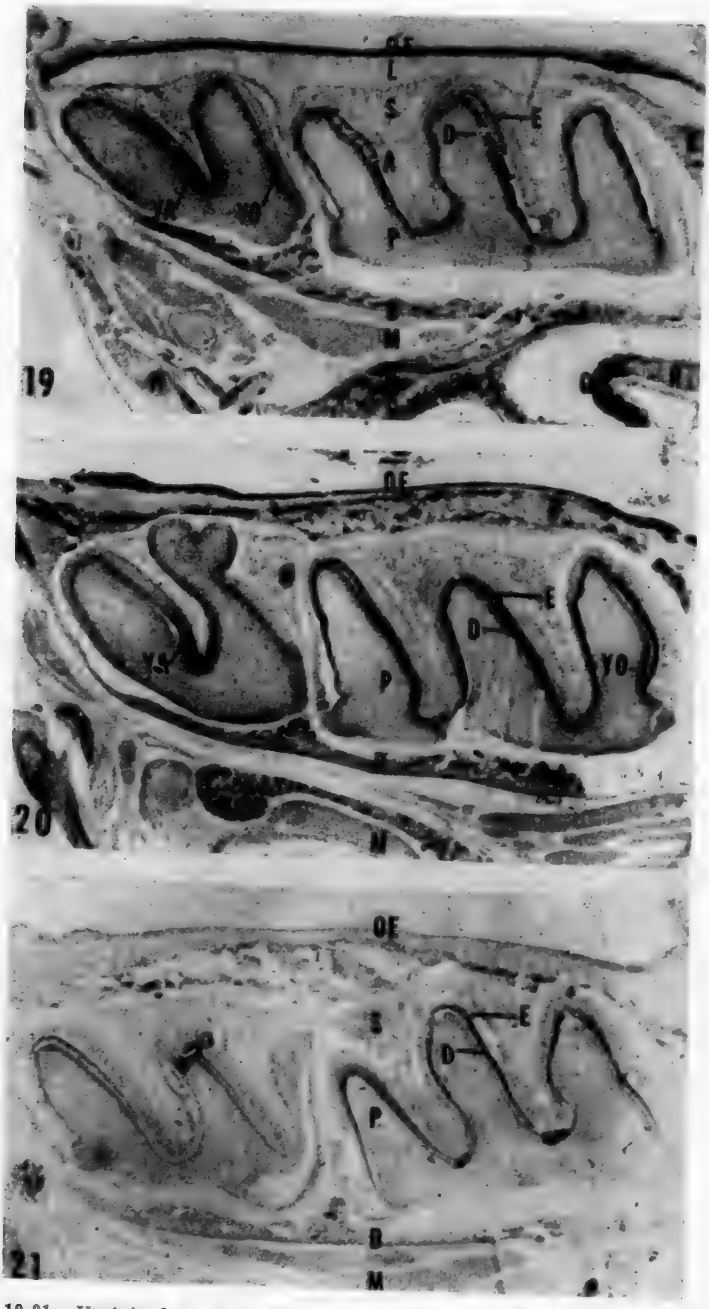


Figs. 10-14.—H-E stained coated radioautographs of cusp of the first molar of rats given C^{14}-glucose at 3 days of age. The teeth were undecalcified (except at the 14-day interval, Fig. 14). Exposure for 3.5 months. Only one side of the cusp is visible in the pictures. (Mag. ×90.) Fig. 10, 3-hour interval (upper molar). Fig. 11, 1-day interval (upper molar). Fig. 12, 3-day interval (lower molar). Fig. 13, 6-day interval (lower molar). Fig 14, 14-day interval (lower molar). *(Continued on opposite page.)*



Figs. 15-18. PA-Schiff stained, coated radioautographs of single cusps of decalcified first molar teeth of rats given C^{14}-glucose at 3 days of age. Exposure for 7 months. Both sides of the cusps are visible. (Mag. ×90.) Fig. 15, 1-month interval (same as Fig. 6). Fig. 16, 2-month interval (same as Fig. 7). Fig. 17, 4-month interval (same as Fig. 8). Fig. 18, 6-month interval (same as Fig. 9). The dentinal reactions (ascending arrows) are intense. Note distance from the occlusal surface to the pulp (*P*).

Figs. 10-14.—*Continued.* Ascending arrows point to the dentinal reaction at the various time intervals. At first (Fig. 10), the reaction is near the tip of the odontoblasts (*O*) at the periphery of the pulp (*P*). Later (Figs. 11-14), the reaction is well within dentin (*D*), while the predentin is free of reaction. The descending arrows point to reactions in the enamel (E). The reactive band is distinguishable in Figs. 10-12 but in Fig. 13 only an exceedingly weak reaction could be seen under high magnification.



Figs. 19-21.—Unstained coated radioautographs of the developing upper first (right-hand side of picture) and second (left) molar of rats given C^{14}-mannose at 3 days of age. Exposure for 4 months. (Mag. ×19.) Fig. 19, 3-hour interval. Fig. 20, 1-day interval. Fig. 21, 3-day interval. *(Continued on opposite page.)*

ameloblast layer (Fig. 19, *A*) produced a reaction, which involved the whole cell and was maximal in the young ameloblasts located near the base of the cusps (Fig. 1, *YA*). The reaction of the young ameloblasts was particularly intense in the second molar bud, which is less advanced in development than the first one (Fig. 19, *YA*). The ameloblastic reaction decreased markedly with time and by 1 month had disappeared, except over the nuclei of some ameloblasts present in the areas which had initially been very reactive (Fig. 25, arrow *A*). A few nuclei in the stratum intermedium also remained reactive (Fig. 25, arrow *S*).

At the early intervals, the pulp of the first molar bud gave a slight reaction (Fig. 19, *P*) and that of the second molar bud a more pronounced reaction (Fig. 19, left-hand side), both of which decreased with time (Fig. 21). The odontoblasts in contrast gave no significant reaction, except for the young ones present at the base of the cusps (Figs. 1, 19, and 20, *YO*). This particular reaction decreased only slightly with time and was recognized at all time intervals (Fig. 26), even at 6 months, particularly in the odontoblasts of the second molar. While the cytologic location of the silver granules was not definitely established, they seemed to be present over both nucleus and cytoplasm.

Alveolar bone reactions predominated on the outer surface of the bony trabeculae surrounding molar teeth (Fig. 1, *B*), that is, on the surface where osteoblasts were abundant. At 3 days, the reactive area approached the inner surface of the alveolar bone (Fig. 3, *B*) as resorption caused this surface to be displaced outward. Later, reactions were no longer seen over the alveolar bone (Figs. 4 and following) and, therefore, the reactive areas themselves must have been removed by erosion. Similarly, in long bones, the labeled material taken up on the outer surface in the center of the diaphysis was found within the matrix by the third day and was later removed by endosteal attrition. By 1 to 6 months, almost all bony reactions had been removed by resorption of the areas of bone containing them. Very few reactive areas escaped resorption and persisted throughout the experimental period (Fig. 27).

Other tissues displayed intense reactions at 3 and 24 hours, but gradually lost their content of radioactivity thereafter. Tissues obtained at the 2-month interval were unreactive in preparations exposed for 7 months, except for the

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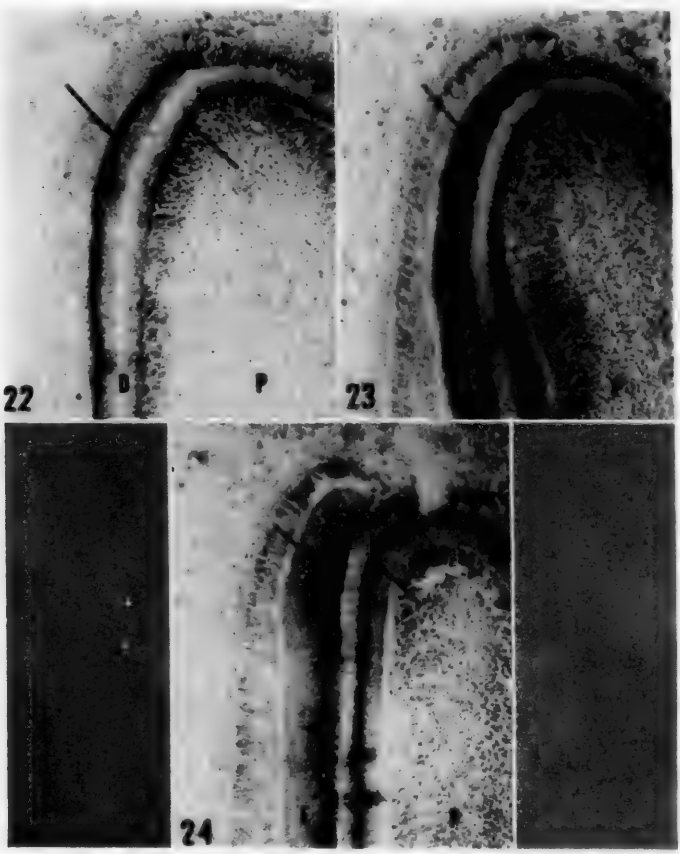
Figs. 19-21.—*Continued.* The first molar clearly shows the dentinal (*D*) and enamel (*E*) reaction bands at the 3 time intervals. The intensity of the enamel reaction is decreased at 3 days (Fig. 21, *E*). The second molar is just starting to develop and the reactions of enamel and dentin are less readily distinguishable than in the first molar.

Fig. 19 shows an intense reaction of deeper layers of oral epithelium (*OE*), a weak reaction of lamina propria (*L*), a moderate reaction of stellate reticulum (*S*) and ameloblasts (*A*), enamel and dentin with their respective reaction bands located in pre-enamel (*E*) and predentin (*D*), the pulp (*P*) which is more deeply reactive in the second than in the first molar, alveolar bone (*B*) mainly reactive on the surface away from the tooth (*B*), muscle (*M*), dot-like reactions in cartilage (*C*) corresponding to individual chondrocytes, mucous (*G*) and serous (*R*) glands. Fairly intense reac ions are observed over young ameloblasts (*YA*) and young odontoblasts (*YO*).

These reactions are similar at 1 day (Fig. 20) with a few differences: the reaction of the oral epithelium (*OE*) predominates in the superficial layers; the enamel reaction band (*E*) is broader, the alveolar bone reaction (*B*) predominates on the surface nearer to the tooth; the mucous glands are no longer reactive, but there is still a reaction in a lachrymal gland (*K*).

By the third day (Fig. 21), the intense reaction of the oral epithelium (*OE*) is lost; reactions of lamina propria, ameloblasts, stellate reticulum (*S*) and pulp (*P*) are decreased; reaction of enamel (*E*) is quite weak, but that of dentin (*D*) is not significantly reduced; rare reactive patches are visible in bone (*B*). Other soft tissues react weakly.

following structures: a few scattered nuclei (particularly those in the outer nuclear layer of the retina), the lens fibers, some of the cartilage (trachea, epiphyses), collagenous fibers in a few connective tissue areas, particularly in the lamina propria of tongue, esophagus, and bladder and in some tendons. Some of the muscles also produced a reaction. Other soft tissues were unreactive.

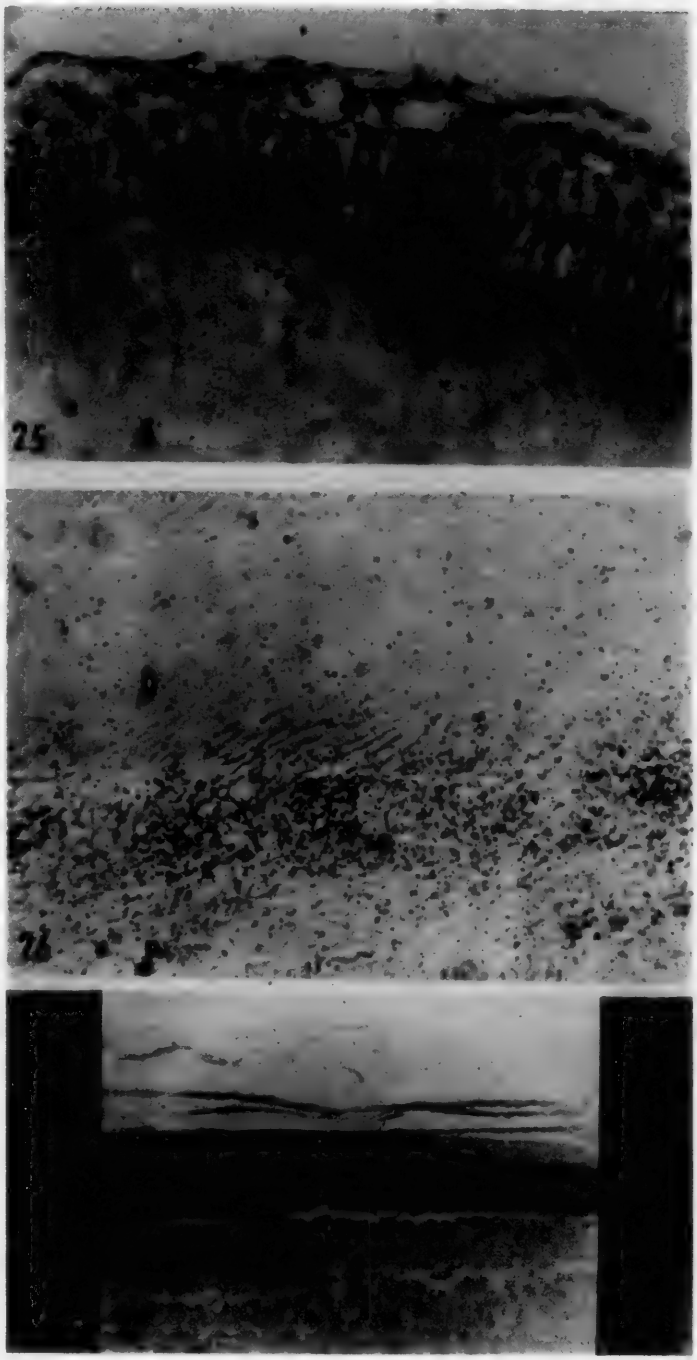


Figs. 22-24.—H-E stained coated radioautographs of the first cusp of the first upper molar of rats given C^{14}-mannose at 3 days of age. Exposure for 4 months. (Mag. ×86.) Fig. 22, 3-hour interval (same as Fig. 19). Fig. 23, 1-day interval (same as Fig. 20). Fig. 24, 3-day interval (same as Fig. 21). The band reaction appearing in pre-enamel (Fig. 22, descending arrow) gradually decreases with time (Figs. 23 and 24). The band reaction appearing in predentin (Fig. 22, ascending arrow) later is seen in dentin (Figs. 23 and 24). The intensity of the reaction reaches a maximum at 1 day (Fig. 23) and remains unchanged at 3 days (Fig. 24).

Fig. 25.—H-E stained coated radioautograph of stratum intermedium (*S*), ameloblasts (*A*) and enamel (*E*) from an animal sacrificed 1 month after C^{14}-glucose injection. High power. Note the reaction on some of the ameloblasts nuclei (arrow, *A*) and on a few rare nuclei in the stratum intermedium (arrow, *S*).

Fig. 26.—Unstained coated radioautograph of dentin (*D*), odontoblasts (*O*), and pulp (*P*) from an animal sacrificed 2 months after C^{14}-glucose injection. High power. Note the reaction of the odontoblasts (arrow).

Fig. 27.—PA-Schiff stained coated radioautograph of shaft of tibia (*B*). The bone marrow (*M*) may be seen below the bone. Low power. An oblique reaction band is visible.



Figs. 25-27.—*For legend see opposite page.*

DISCUSSION

Chemical Nature of the Radiocarbon Taken Up in Hard Tissues.—The radioautographic reactions observed 3 hours to 6 days after administration of C^{14}-glucose were not affected by staining with hematoxylin although, in our experience, the acid present in the stain removes minerals from sections of teeth of young rats. Furthermore, the tissues obtained at the 14-day and later intervals had been decalcified. Therefore, the labeled materials observed in the radioautographs were not in a mineral but in an organic form. Since this material was retained during histologic procedure, it may be presumed not to consist of soluble substances, such as the free hexoses themselves or their metabolic products (pyruvate, bicarbonate, etc.), but rather of substances of high molecular weight, such as the protein collagen and the smaller amounts of carbohydrates known to exist in dentin.[1-6] A priori, it was not possible to state whether hexoses were taken up as such in these large molecules or were first broken down into metabolites (e.g., bicarbonate) which were then synthesized into large molecules.

A partial answer was supplied by a comparison with radioautographs obtained at various time intervals after administration of C^{14}-bicarbonate.[12] In dentin, the pattern observed under these conditions was indistinguishable from that described above for C^{14}-glucose. In enamel, however, only a negligible reaction was produced with C^{14}-bicarbonate in contrast to the intense one arising after administration of C^{14}-glucose (Figs. 1-3).

Like dentin, most soft tissues of the experimental animals showed a distribution of the C^{14} from glucose similar to that of the C^{14} from bicarbonate (Kumamoto[13]). An exception was the reaction of intestinal goblet cells which, like that of enamel, occurred only after labeled glucose administration.

Since the enamel reaction was observed with C^{14}-glucose but not with C^{14}-bicarbonate, it must result from the uptake of glucose itself or of a glucose metabolite which is not readily synthesized from bicarbonate. Perhaps glucose enters the polysaccharide which Nikiforuk[8] has shown to be present in enamel. If so, the finding of Schiller, Mathews, Cifonelli, and Dorfman[11] that glucose enters the hexosamine and probably the glucuronic acid residues of acid mucopolysaccharides would apply to the carbohydrates of enamel.

The dentinal reaction, which was similar after labeled glucose or bicarbonate, may be due to the incorporation of a common metabolite of these two substances, which may in fact be bicarbonate itself, since glucose is readily degraded to carbon dioxide.[15] The entry of the C^{14} from glucose into dentin as bicarbonate or as a common metabolite of the 2 substances is supported by the appearance of the C^{14} label of glucose in predentin (Fig. 10), a region which histochemical tests show to be poor in carbohydrates,[12] whereas the uptake of glucose as a precursor of carbohydrates would have been likely to occur at the predentinodentinal junction, where the deposition of S^{35}-sulfate indicates carbohydrate synthesis.[16]

The radioautographic reactions observed after administration of labeled mannose (Figs. 21-24) were qualitatively identical to those observed after

labeled glucose (Figs. 10-12), although glucose had all its carbons labeled and mannose, only the 1 position. The similarity of the dentinal reactions may be due to the fact that mannose like glucose was degraded to bicarbonate or related metabolites. The identity of the pattern observed in enamel with the 2 hexoses was more surprising. Perhaps, mannose transformed into glucose. Perhaps, enamel utilized both hexoses indiscriminately for the polysaccharide synthesis assumed to occur in this tissue.

Conclusions Regarding Growth of Teeth.—The radioautographic pattern observed soon after administration of C^{14}-glucose was characterized by the appearance of bands in enamel (e.g., Fig. 1) and dentin (e.g., Fig. 11). These bands were, in fact, sections of layers of labeled material, which initially arose in close contact with the apices of the ameloblasts in pre-enamel and of the odontoblasts in predentin. Such observations provide a direct demonstration of the accepted belief that these cells elaborate layers of their respective matrices. Furthermore, since identical observations were made at whatever time of the day the animals had been given C^{14}-glucose, it may be concluded that the addition of material to the matrix proceeds continuously in both tissues.

The accretion of matrix may be shown in another way. With time, the circulating C^{14}-glucose decreased and was replaced by unlabeled glucose and, consequently, newly formed matrix also was unlabeled. This process eventually produced a separation of the labeled layer from the matrix-producing cells by an increasingly thick layer of unlabeled matrix (Figs. 1-9).

As the labeled layer was displaced from the matrix-producing cells, it underwent the changes postulated classically. Thus, in the tooth, predentin became converted into dentin, pre-enamel into young and transitional enamel; and, during these transformations, both matrices acquired the ability to calcify (abruptly at the predentino-dentinal junction and gradually in the young and transitional enamel).

Later, the development of the matrices differed markedly. In the enamel organ, the reactive material, on reaching the zones of calcification, diminished gradually, due either to a true departure of labeled material or perhaps to its dilution by the considerable deposition of minerals taking place. In contrast, the labeled materials laid down in dentin were retained for at least 6 months after radioglucose administration (Figs. 10-18). Except at the occlusal surface where dentin is worn away by attrition, the dentinal matrix seems to persist indefinitely. The stability of the dentinal reactions was all the more striking when compared to the gradual decrease in the reactions of most soft tissues.

However, discrete persistent reactions were seen not only in dentin, but also in bone matrix (Fig. 27), in the lens and, to a lesser degree, in muscle, cartilage and accumulations of collagenous fibers (laminae propriae, tendon, etc.), as well as in ameloblasts (Fig. 25) and odontoblasts (Fig. 26). It may be speculated that, of the organs and tissues examined, 2 main types of structures contain permanent components derived from glucose: (1) nuclei, such as those of ameloblasts, odontoblasts, and retina, which presumably had been formed by mitosis soon after the injection of radioglucose (and perhaps did not divide

thereafter)—a result supporting classical views on the stability of DNA,[17] and (2) dentin and bone matrix, cartilage, and collagenous fibers (lamina propria of esophagus, tongue, bladder). Since the matrices of dentin and bone, as well as cartilage and collagenous fibers, are known to be rich in collagen,[4, 18, 19] it is suggested that the persisting component in all these structures is collagen synthesized soon after the injection of labeled glucose. Thus, of the numerous substances which arise from glucose metabolism, 2 appear capable of permanent stability: desoxyribonucleic acid and collagen. Radioautography of the tooth illustrates both cases.

SUMMARY

The fate of glucose uniformly labeled with C^{14} was investigated in 3 groups of rats injected 3 days after birth and sacrificed between 3 hours and 6 months later. Teeth and associated tissues were examined using the coating method of radioautography. In addition, a group of rats given mannose-1-C^{14} was examined in the same manner. The distribution of labeled glucose and mannose was qualitatively indistinguishable.

In the enamel organ, the radioactive carbon of glucose is taken up into the pre-enamel matrix where it is arranged as a layer in close contact with the apex of the ameloblasts. During the next few days, as new pre-enamel is formed, the radioactive layer is seen within the zones of calcification (young and maturing enamel) and appears broader and less intense. With time, the radioactivity is no longer detectable. The available evidence indicates that labeled glucose has been taken up into large-sized molecules (presumably carbohydrates) of the pre-enamel matrix, which may be turned over rapidly.

In the dentinal organ, the radioactive carbon of glucose is taken up into the predentinal matrix, where it is arranged as a layer in close contact with the apex of the odontoblasts. As new predentin is continually deposited, the radioactive layer appears in the calcified zone, that is, in dentin proper. The labeled layer remains at a constant distance from the dentinoenamel junction and retains the same intensity throughout the 6-month experimental period.

The rapid decrease in the widespread radioautographic reactions observed in enamel and most soft tissues is in contrast to the stability of the dentinal reactions which persist indefinitely unless eroded by attrition. Similarly, stable material has been found in bone matrix, cartilage, and collagenous fibers. It is suggested that collagen is the common stable element present in these tissues.

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THE INTERMEDIATE BIFURCATIONAL RIDGE: A STUDY OF THE MORPHOLOGY OF THE BIFURCATION OF THE LOWER FIRST MOLAR

FRANK G. EVERETT, M.S., D.M.D., M.D., ELLIS B. JUMP, D.M.D., PH.D., THOMAS D. HOLDER, B.A., D.M.D., AND GEORGE C. WILLIAMS, D.M.D.

(The Dental School of the University of Oregon, Portland, Ore.)

THE observation of certain irregularities in the exposed bifurcation of the lower first molars has led to an investigation of these features. A study of the bifurcation of 328 extracted lower molars, divided into 3 groups, was undertaken. In 107 of these teeth (Group 1), buccolingual specimens were prepared by grinding off the mesial half of the tooth, preserving the distal half up to the height of the bifurcation. In 112 other of these teeth (Group 2), the distal half of the tooth was sacrificed, preserving the mesial half of the tooth up to the height of the bifurcation. In 109 teeth (Group 3), both roots were amputated at right angles at a line about 2 mm. from the height of the bifurcation. All these specimens were studied under a dissecting microscope (×18). Buccolingual ground sections ground at the height of the bifurcation were then prepared of 35 molars from Group 3. An additional 20 teeth from Group 3 were decalcified and embedded in celloidin. Ten of them were sectioned serially in a buccolingual direction through the bifurcation, the other 10 were sectioned serially in mesiodistal direction. All decalcified sections were stained with hematoxylin and eosin. Every effort was made to observe, as closely as possible, a correct buccolingual plane in cutting and sectioning of gross specimens, ground sections, and serial decalcified sections, in order to avoid possible tangential views of the roots.

RESULTS

The most striking feature found in a majority of specimens was the presence of a distinct ridge, running across the bifurcation in a mesiodistal direction. We called this *the intermediate bifurcational ridge* (Figs. 1-4, 6-11). This ridge, where present, originates on the mesial surface of the distal root about 2 mm. from the height of the bifurcation, runs across the bifurcation, and ends high up on the mesial root, there blending into the concavity characteristic for the distal surface of the mesial root of this tooth (Fig. 4). This intermediate ridge was found pronounced (Figs. 1, 2, 4, and 6) in 44 per cent, noticeable (Fig. 3) in 29 per cent, and absent (Fig. 5) in 27 per cent of the teeth examined (Table I).

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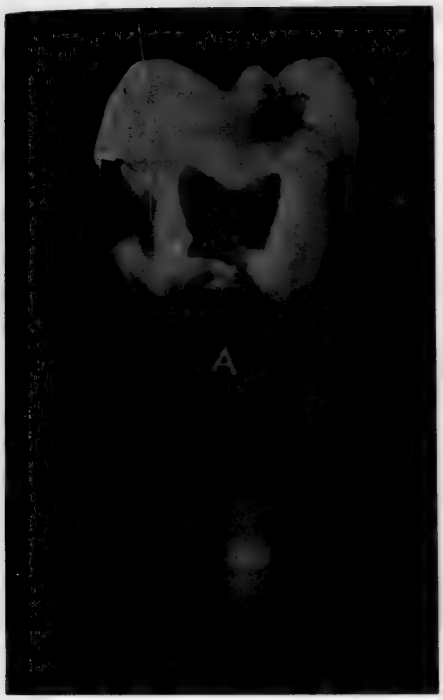

Fig. 1.

Fig. 2.

Fig. 1.—Mesial root of mandibular first molar sectioned through the height of the bifurcation, showing (*A*) the intermediate bifurcational ridge.

Fig. 2.—Distal half of mandibular first molar, showing cross-section (*A*), and origin (*B*) of the intermediate bifurcational ridge.

Fig. 3.—Distal half of mandibular first molar, showing the presence of bifurcational concavity (*A*) demarcated by buccal (*B*) and lingual (*C*) bifurcational ridges.

TABLE I

	PRESENCE OF INTERMEDIATE BIFURCATIONAL RIDGE						AT THE HEIGHT OF THE BIFURCATION IN A BUCCOLINGUAL DIRECTION CAN BE NOTED A:						IS THE LINGUAL OR THE BUCCAL BIFURCATIONAL RIDGE THE MORE ACUTE?					
	PRONOUNCED		NOTICEABLE		ABSENT		CONCAVITY		CONVEXITY		PLANE		BUCCAL MORE ACUTE		LINGUAL MORE ACUTE		NO. DIFF. NOTICEABLE	
	NO. OF TEETH	PER CENT	NO. OF TEETH	PER CENT	NO. OF TEETH	PER CENT	NO. OF TEETH	PER CENT	NO. OF TEETH	PER CENT	NO. OF TEETH	PER CENT	NO. OF TEETH	PER CENT	NO. OF TEETH	PER CENT	NO. OF TEETH	PER CENT
Group 1 (distal root) 107 teeth	54		24		29		14		38		55							
Group 2 (mesial root) 112 teeth	47		38		27		21		44		47							
Group 3 (amputation) 109 teeth	43		33		33		14		46		49		44		25		40	
Total — 328	144	44	95	29	89	27	49	15	128	39	151	46	44	40	25	23	40	37

Next, the type of surface in the bifurcation from which the intermediate ridge rose was studied. In 15 per cent of the teeth examined, the intermediate ridge projected above a buccolingual concavity (Fig. 3), in 39 per cent the ridge projected from convex bifurcational surfaces (Fig. 2), while in the remainder (46 per cent), this inter-radicular area was flat (Fig. 7 and Table I).

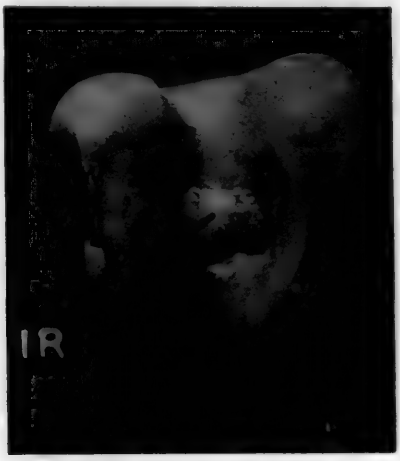

Fig. 4.—Mandibular first molar with amputated roots, showing the presence of an intermediate bifurcational ridge (*IR*). See Text.

Fig. 5.—Mandibular first molar with amputated roots, showing a bifurcational concavity and a pronounced lingual ridge (*A*).

Where a buccolingual plane or concavity was present in the bifurcation, it was delineated on its buccal and lingual borders by prominent line-angles between the respective sides of the tooth and the bifurcational surface. These prominent borders were designated the buccal or lingual bifurcational ridges.

The buccal bifurcational ridge was found more acute than the lingual one in about 40 per cent of teeth (Fig. 3). In 23 per cent of teeth, the lingual ridge was more acute than the buccal ridge (Figs. 5 and 7). There was no

noticeable difference between the 2 ridges, or these ridges were not apparent in the remaining 37 per cent of cases (Fig. 6 and Table I). For the percentile determination of these last figures, we used only the 109 specimens of Group 3

Fig. 6.—Buccolingual ground section of mandibular first molar. Pronounced intermediate bifurcational ridge. Possible enamel layer (*A*) covered by bifurcational ridge. Dentin (*D*) extends into bifurcational ridge.

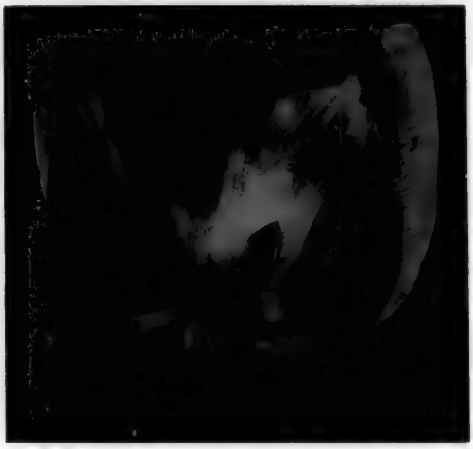

Fig. 7.—Buccolingual ground section of mandibular first molar photographed by reflected light. Intermediate bifurcational ridge.

with intact bifurcations in which both roots had been amputated at right angles at a line about 2 mm. from the height of the bifurcation, and relied upon examination with a magnifying glass and under the dissecting microscope (Figs. 4 and 5). Buccolingual serial sections of decalcified specimens had demonstrated the difficulty, even with best of care, of obtaining correct information as to the acuteness or obtuseness of the buccal and lingual bifurcational

ridges from sectioned material. One and the same tooth, sectioned serially, would show the buccal ridge more acute in some sections and the lingual one in others.

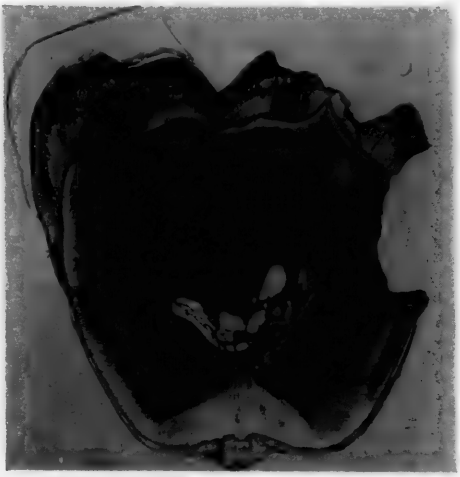

Fig. 8.—Buccolingual ground section of mandibular first molar. Prominent intermediate bifurcational ridge.

Fig. 9.—Detail of Fig. 8 under higher magnification. Note borders (*BC*) of the basilar cone of dentin from which the bifurcational ridge arises, zone of dislocation (*ZD*) extending across the basilar cone, and the core of dentin (*D*) upon which the bifurcational ridge has formed.

HISTOLOGIC FINDINGS

Study of buccolingual ground sections from 35 molars indicates that the intermediate ridge is formed primarily by cementum (Figs. 6-9). However,

as can be seen on some gross sections (Fig. 1), and the microscopic films (Figs. 6-11), this ridge has a basis in dentin on which the extensive cementum deposition occurs. The buccal and lingual ridges, on the other hand, are essentially dentin formations covered with only a small amount of cementum (Figs. 7, 8, 10, and 11).

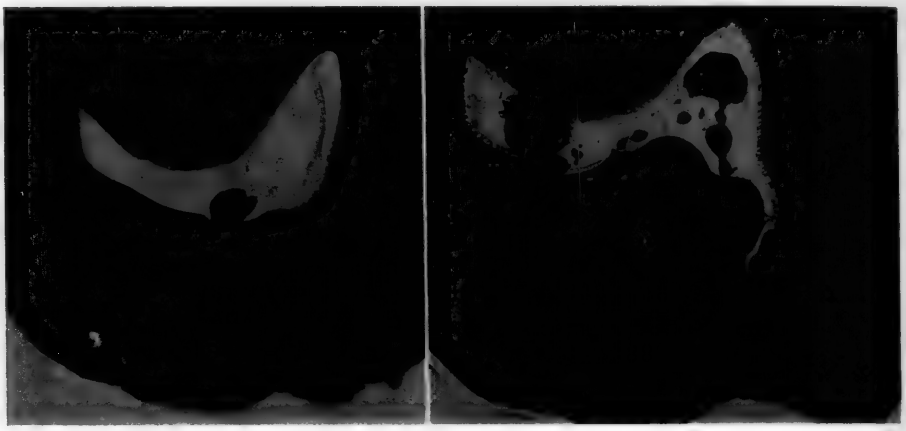

Fig. 10. Fig. 11.

Fig. 10.—Buccolingual celloidin section of mandibular first molar. Hematoxylin and eosin stain. Basilar cone of dentin forming plug in floor of pulp chamber and extending into base of bifurcational ridge. Vascular channel (*A*) at border of basilar cone.

Fig. 11.—Buccolingual celloidin section of mandibular first molar. Hematoxylin and eosin stain. Note elevated buccal bifurcational ridge, intermediate bifurcational ridge, and basilar cone with zone of dislocation and vascular channels at its borders.

Twenty teeth from Group 3 were decalcified and embedded in celloidin. Ten such teeth were sectioned serially in a buccolingual direction and the remaining 10 in a mesiodistal direction. All were stained with hematoxylin and eosin. Findings were similar to those obtained from ground sections. With the exception of 1 ground section (Fig. 6), neither the ground sections nor the decalcified material showed any indications of the presence of enamel[1, 4] in the bifurcation to explain the occurrence of the bifurcational ridge. The dentin cone forming the base of the pulp chamber appeared different histologically from the rest of the dentin in both ground and decalcified material. This part of the tooth may be exposed to certain unknown hazards during formation, accounting for this difference in structure.[3]

This cone always presented its apex upward as a plug in the floor of the pulp chamber, and its widely flaring sides reached the bifurcational surface somewhat buccal or lingual to the points where the intermediate ridge projected apically. Often nutrient canals were noted at the borders of this dentinal cone.[1, 4] Invariably this cone also exhibited a transverse zone of dislocation of dentinal tubules which resembles the familiar break between primary and true secondary dentin of the tubular type (Figs. 6, 7, and 10).

DISCUSSION

It has been known, of course, that cementum deposits are quite heavy in the bifurcation of multirooted teeth,[5] but it is felt that the intermediate bifurcational ridge deserves attention from both the descriptive anatomic and from the clinical periodontal viewpoints. The presence of bifurcational concavities, and of buccal and lingual bifurcational ridges seems noteworthy for the same reasons. What, if any, influence age or disease has on the development of the intermediate bifurcational ridge, we do not know. It might be assumed that this, like other cemental structures, would develop with advancing age. However, as can be seen from a comparison of Figs. 1 and 2, showing young teeth with large pulp chambers and a pronounced intermediate ridge, with Fig. 3, showing an older tooth with smaller pulp chamber and a smaller intermediate ridge, this relationship is not always evident.

CONCLUSIONS

1. The bifurcation of 328 extracted lower first molars has been examined, under the dissecting microscope, by the study of ground sections and in decalcified, celloidin-embedded serial sections.

2. An anatomic structure, hitherto undescribed, the intermediate bifurcational ridge, has been noted in 73 per cent of 328 lower first molars.

3. Other structures described are the buccal and lingual bifurcational ridges, guarding, as it were, the entrance to the bifurcation of the lower first molar and determining the ease of access to the exposed bifurcation from either buccal or lingual direction. The frequency of their occurrence is noted.

4. Frequencies of bifurcational buccolingual concavity, convexity, and plane are also described.

The authors acknowledge the assistance of Stephen M. Clark in the collection and preparation of the specimens.

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THE EFFECT OF VARYING LEVELS OF FLUORIDE ON DENTAL CARIES IN SALIVARIADENECTOMIZED RATS

JOSEPH C. MUHLER

Indiana University, Bloomington, Ind.

PREVIOUS work has indicated that fluorides are effective in reducing the incidence of dental caries in salivariadenectomized rats.[1, 2] However, the period during caries development when the fluoride acts is not known, nor is it known whether fluoride affects caries initiation or progression differently. The solution to this is of importance since it will help provide evidence concerning the mechanism of fluoride action in the rat. Salivariadenectomized rats were used in this investigation due to the rapid progression of the carious lesions in these animals. Furthermore, evidence from this laboratory[3] has suggested that the absence of saliva does not affect caries initiation in the rat in the absence of initiating factors, but does markedly increase caries progression.

EXPERIMENTAL

This study consists of two series. Series I was composed of 100 weanling rats (Sprague-Dawley strain) divided equally into 2 groups according to sex and initial body weight. Group 1 was salivariadenectomized by procedures already described[4] and received stannous chlorofluoride placed in their drinking water at a fluoride concentration of 30 μg per milliliter. Group 2 was salivariadenectomized and received fluoride-low drinking water (F = 0.05 μg/ml.). After 50 days, one half of each group was sacrificed by ether and their heads were studied for dental caries by procedures already described.[5] The balance of the animals remained on the study for an additional 50 days at which time they were similarly sacrificed and their heads were examined for dental caries.

Series II was composed of 250 weanling rats (Sprague-Dawley strain) divided equally into 2 groups according to sex and initial body weight as in Series I. Group 1 was salivariadenectomized and received stannous fluoride placed in the drinking water at a fluoride concentration of 50 μg per milliliter. Group 2 was salivariadenectomized and received the same fluoride-low drinking water as the animals in Group 2 of Series I. After 25, 50, 75, 100, and 125 days, 25 rats from each of the two groups were sacrificed by ether and the heads were removed for dental caries examination.

All of the animals in both series received the same stock corn cariogenic diet, the composition of which has previously been reported.[6] All of the animals were housed in pairs in raised screen cages in an air-conditioned room.

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DATA AND DISCUSSION

The data for Series I and II are found in Tables I and II, respectively. In the salivariadenectomized group not receiving stannous chlorofluoride there were 8.0 lesions after 50 days and 9.4 lesions after 100 days. The cariogenic effects produced by salivariadenectomy are so severe that after a period of only 50 days the greater incidence of dental caries had already occurred. The animals which received the stannous chlorofluoride had numerically, but not significantly, less dental caries after 50 days, and significantly less caries at the end of the 100-day period.

TABLE I
THE EFFECT OF STANNOUS CHLOROFLUORIDE ON DENTAL CARIES IN SALIVARIADENECTOMIZED RATS

	PERIOD ON EXPERIMENT FOLLOWING SALIVARIADENECTOMY (IN DAYS)			
	50		100	
GROUP	NO. ANIMALS	MEAN NO. LESIONS	NO. ANIMALS	MEAN NO. LESIONS
Salivariadenectomized + H_2O	18	8.0	19	9.4
Salivariadenectomized + SnClF	20	6.5	22	7.7
P value		0.10		0.05

TABLE II
THE EFFECT OF STANNOUS FLUORIDE ON DENTAL CARIES IN SALIVARIADENECTOMIZED RATS SACRIFICED AT VARIOUS TIME INTERVALS

	PERIOD ON EXPERIMENT FOLLOWING SALIVARIADENECTOMY (IN DAYS)									
	25		50		75		100		125	
GROUP	1*	2*	1	2	1	2	1	2	1	2
Salivariadenectomized + H_2O	24	4.1	24	6.2	21	8.7	24	9.2	21	10.0
Salivariadenectomized + F†	24	2.5	24	4.7	21	6.5	24	6.9	27	7.1
P value		0.03		0.06		0.05		0.01		0.005

*Column 1 indicates the number of animals in the group; Column 2 indicates the mean number of carious lesions.

†All animals received 50 ppm of fluoride as stannous fluoride from a solution prepared fresh daily.

The data obtained in Series II corroborate the observations obtained in Series I in regard to the effect of a tin fluoride on dental caries. In this series, stannous fluoride was used in place of the stannous chlorofluoride used in Series I, and also a higher fluoride concentration was placed in the drinking water. The anticariogenic effectiveness of the fluoride is evident at each of the periods the animals were sacrificed, but the effect becomes more dramatic after the 75-day period. This may be a result of the fact that the dental caries experience in the salivariadenectomized animals not receiving fluoride continues to increase throughout the study while the animals receiving stannous fluoride have about the same caries experience after 75 days. These data are of interest since it is a clinical impression obtained by examining children, previously treated with

aqueous solutions of stannous fluoride, that this compound has a pronounced inhibitory effect on caries already present in the mouth at the time of the initial treatment. However, additional animal studies need to be conducted, and human topical studies designed to investigate this problem are necessary in order to learn more about this important clinical observation.

As a result of these studies, it was of interest to compare the anticariogenic effectiveness of stannous fluoride and sodium fluoride in salivariadenectomized rats. The results of such a study are seen in Table III. The results suggest that stannous fluoride at high concentrations is not as effective as at lower concentrations, while sodium fluoride shows an inverse relationship between fluoride concentration and the dental caries experience. Not being able to explain these data in regard to stannous fluoride, it was felt necessary to repeat and expand this phase of the problem. A total of 210 male weanling Sprague-Dawley strain rats were divided into 7 groups as shown in Table IV. Two stannous fluoride samples from 2 different sources were used, since in vitro and in vivo animal data have suggested the possibility of minor biologic differences in stannous fluoride preparations from different sources. Daily food and water consumption were obtained on each of the groups throughout the 140-day experimental period. The animals were housed in the same manner and received the same diet as the animals used in Series I and II. The animals were not salivariadenectomized. Every other day throughout the study the solutions were analytically checked for the proper fluoride concentration in each of the groups, and if any variation beyond ± 1.0 at the 10 ppm fluoride level, ± 3.0 ppm at the 30 ppm level, and ± 10 ppm at the 100 ppm level occurred, the solutions were adjusted to correct for these differences.

TABLE III
A COMPARISON BETWEEN SODIUM FLUORIDE AND STANNOUS FLUORIDE AT 3 DIFFERENT FLUORIDE LEVELS ON DENTAL CARIES IN SALIVARIADENECTOMIZED RATS

FLUORIDE GROUP	F CONC. (PPM)	NO. OF ANIMALS	MEAN NO. OF LESIONS
NaF	25	19	8.1
SnF_2		24	6.0
NaF	50	18	6.9
SnF_2		22	8.1
NaF	100	20	4.2
SnF_2		20	6.8

The results of this study are found in Table IV. These data clearly indicate a decrease in dental caries with increasing fluoride concentrations for both stannous fluoride preparations. While there may be minor differences in the anticariogenic effectiveness of the 2 different stannous fluoride preparations used in this study, these are not considered to be biologically important. Of considerably more interest are the differences in food and water consumption which occur with increasing fluoride concentration. The SnF_2 sample A at the 10 ppm level results in less dental caries than the SnF_2 sample B. However, the animals receiving the SnF_2 sample A and the sample B consumed approximately the same amount of fluoridated water, but ate considerably different

TABLE IV
A COMPARISON OF THE ANTICARIOGENIC EFFECTIVENESS OF 2 SAMPLES OF STANNOUS FLUORIDE, ALONG WITH THE AMOUNT OF DIET EATEN AND DRINKING WATER CONSUMED DAILY

GROUP	NO. OF ANIMALS	FLUORIDE CONC. (PPM)	MEAN NO. OF LESIONS	P	MEAN DAILY DRINKING WATER CONSUMED (ML.)	MEAN DAILY FOOD CONSUMPTION (GM.)
Control	23	–	8.6	–	20.6	28.4
SnF_2 A*	27	10	6.5	0.07	19.5	25.4
SnF_2 B†	22	10	8.0	0.4	20.2	31.1
SnF_2 A	28	30	6.1	0.04	15.9	25.0
SnF_2 B	26	30	6.5	0.05	19.5	28.1
SnF_2 A	23	100	4.9	0.01	12.6	27.5
SnF_2 B	24	100	5.5	0.01	11.0	25.8

*Metal and Thermit preparation.
†Indiana University preparation.

amounts of the cariogenic diet. The animals receiving the SnF_2 sample B ate approximately 5 Gm. more food per rat per day than the animals receiving the SnF_2 sample A.

At the 30 ppm fluoride level the animals receiving the SnF_2 sample A drank approximately 5 ml. less fluoridated water than those receiving SnF_2 sample A at the 10 ppm level, while the food consumption was approximately identical. However, the animals receiving the SnF_2 sample B drank about 4 ml. a day more fluoridated water and ate about 3 Gm. more food than those receiving the SnF_2 sample A at the same fluoride level. These data would seem to indicate that even though the animals receiving the SnF_2 sample B received more fluoride, the increased food consumption was more important a cariogenic agent than the fluoride was an anticariogenic agent.

At the 100 ppm fluoride level, the pronounced difference in water consumption is evident. For both fluoride samples used, the animals drank significantly less fluoridated water than any of the other groups. At this fluoride level, the caries reduction must be related to the increased fluoride intake. It is interesting that the higher caries score in the SnF_2 sample B is not associated with the greater food consumption, while at the 10 and 30 ppm levels the caries experience appears to be directly associated with food consumption. Thus, at the lower fluoride levels the cariogenic effect of the diet appears to overcome the anticariogenic effect of the fluoride, while at the higher fluoride levels this does not appear significant and the fluoride begins to overshadow the cariogenic effect of the diet. Based upon previous work[7] which relates food consumption to the dental caries experience in rats, this difference in food consumption between groups could well explain the dental caries differences. These data clearly indicate, as have previous animal studies,[8] that the feeding habits, the amount of diet, and the amount of fluoride received are all important variables in experimental dental caries studies.

CONCLUSIONS

In 2 separate studies, stannous chlorofluoride and stannous fluoride have been shown to reduce significantly the dental caries experience in salivari-adenectomized rats. Increasing the amount of fluoride in the drinking water

as sodium fluoride decreases the dental caries experience, while a similar increase in the fluoride concentration of the drinking water, furnished as stannous fluoride, had no effect on caries. These data may be explained by the study designed to compare the anticariogenic effectiveness of 2 stannous fluoride preparations. Analysis of the data indicated pronounced differences in fluoridated water intake and minor differences in food intake. At a level of 100 ppm fluoride in the drinking water as stannous fluoride, there was approximately a 40 per cent reduction in water consumption when compared to a group receiving 10 ppm fluoride in the drinking water. It is suggested that food intake, water intake, and feeding habits be carefully controlled in experimental dental caries studies.

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ISOLATION OF A BACTERIOPHAGE SPECIFIC FOR A LACTOBACILLUS CASEI FROM HUMAN ORAL MATERIAL

CHARLES E. MEYERS, D.D.S., PH.D., EUGENE L. WALTER,* B.S., AND LYNN B. GREEN, B.S.

Naval Biological Laboratory, Naval Supply Center, and University of California, Department of Bacteriology, Oakland, Calif.

THE classification of the lactobacilli is based upon biochemical, serologic, and morphologic characteristics. Since phage typing has proved useful for the classification of other bacteria, the availability of bacteriophages for various species of lactobacilli would probably expedite and simplify the classification of these organisms. Sierakowski and Zajdel[1] reported the isolation of a "lytic agent" active against a bacterium isolated from a carious tooth. McKinley[2] examined surgical specimens from various body areas and reported isolation of a polyvalent bacteriophage from a filtrate made from a human submaxillary gland removed because of "sialolithiasis with chronic inflammation." Wagemans[3] reported the isolation from human saliva of a bacteriophage active against 3 organisms of the colon-typhoid group (*B. coli, B. voldagsen,* and *B. typhus*). However, the occurrence of bacteriophages in the human oral cavity and their possible relation to oral disease, and the ecology of the oral flora, is unknown. Because dental caries is the most common dental disease, and the lactobacilli are the organisms most commonly thought to be associated with this disease, the occurrence of oral bacteriophages against 12 strains of lactobacillus was investigated.

MATERIALS AND METHODS

Mouth wash specimens were tested for lytic action against 12 strains of lactobacillus (Table I), using essentially the technic of Gardner and Weiser.[4] An aliquot was removed from each specimen and filtered through a Millipore filter (MF).† Four drops (18-gauge needle) of each filtrate were placed on lawns of lactobacilli. As a control, a drop of sterile 1 per cent peptone water was deposited in the center of each plate. Lawns were prepared by dallying approximately 0.2 ml. of a 24-hour culture of each species on separate agar‡ plates and allowing the plates to dry. The plates, after inoculation with filtrate, were incubated (37° C.) and examined for plaques at intervals. The remainder of each specimen was seeded with suspensions of washed concentrated cells of 1

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*Present address: Dept. of Medicine, Univ. of Southern California, Los Angeles 7, Calif.

†Millipore Filter Corporation, Watertown, Mass.

‡Tryptone-glucose-extract agar (Difco) with 0.5 per cent yeast extract added.

TABLE I
SPECIES AND SOURCE OF LACTOBACILLI USED FOR SEEDING MOUTH WASH SAMPLES*

LACTOBACILLUS	SOURCE
Lactobacillus species NLF†	Dr. Lucile Smith, College Physicians and Surgeons, San Francisco, Calif.
L. casei, var. *rhamnosus*	Oral isolate
L. casei	ATCC No. 4646
L. acidophilus	ATCC No. 4356
L. acidophilus	U. of Calif., Dept. Dairy Industry‡
L. acidophilus	N.B.L. stock strain
L. acidophilus	Oral isolate
L. fermenti, No. 1	Oral isolate
L. fermenti, No. 2	Oral isolate
L. fermenti	USDA Agric. Res. Serv. No. 36§
L. salivarius	Oral isolate
L. helviticus	N.B.L. stock strain

*All species of lactobacilli were tested and, where appropriate, reclassified according to the biochemical criteria of Wheater[9] and Rogosa, Wiseman, Mitchell, Disraely, and Beaman.[5] *L. casei*, var. *rhamnosus* and *L. salivarius* were designated according to Rogosa.[5]

†This strain met most of the requirements of *L. casei*, var. *alactosus* as set forth by Rogosa, Wiseman, Mitchell, Disraely, and Beaman.[5]

‡Courtesy of Department of Dairy Industries, University of California, at Davis.

§Kindly supplied by Dr. J. C. Lewis, USDA, Agric. Res. Serv., Albany, Calif.

to 3 of the 12 strains of lactobacillus in sterile distilled water. After 24-hour incubation, the specimens were tested again for bacteriophage as described, reseeded with the species previously employed, and reincubated. The procedure was repeated at 24-hour intervals until bacteriophage was demonstrated, or 5 successive tests were negative. Agar, removed from areas where lysis had occurred, was macerated in 1 per cent peptone solution. This suspension was then enriched with the particular species against which lysis had occurred, incubated from 6 to 24 hours, filtered through MF, and inoculated onto lawns of the same species.

RESULTS AND DISCUSSION

From a pooled mouth wash specimen from 2 of us (C. E. M. and E. L. W.), enriched with a nonlactose fermenting (NLF) lactobacillus species (meeting most of the requirements of *Lactobacillus casei*, var. *alactosus;* Rogosa, Wiseman, Mitchell, Disraely, and Beaman[5]), bacteriophage for this organism was isolated. The bacteriophage was not effective against *L. acidophilus*, ATCC 4356; *L. fermenti*, strain No. 1; *L. fermenti*, strain No. 2; *L. casei*, var. *rhamnosus; L. casei*, ATCC 4646; *L. pentosus; L. delbrueckii; L. helveticus; Leuconostoc citrovorum*, ATCC 7013; *Leuconostoc citrovorum*, ATCC 8081; *Leuconostoc dextranicum*, ATCC 8086; *Serratia marcescens; C. diphtheriae; E. coli; E. coli*, var. *communior; Aerobacter aerogenes; Kleb. pneumoniae; Malleomyces pseudomallei*, NBL No. 118.

Repeated tests have not yet shown the enriching species to be lysogenic. Lytic activity against the lactobacillus species NLF has been demonstrated in 14 of 25 mouth wash specimens examined. Lytic activity has also been demonstrated against 6 additional species of lactobacilli (Table II).

By the agar layer technique of Gratia (Adams[6]), the plaques produced by the lactobacillus species NLF bacteriophage were clear, distinct areas of lysis

TABLE II
DISTRIBUTION OF LYSIS TESTS AMONG 6 LACTOBACILLI

LACTOBACILLUS	NUMBER SPECIMENS PRODUCING PLAQUE-LIKE AREAS
Lactobacillus species, NLF	14
L. casei, ATCC 4646	11
L. casei, var. *rhamnosus*	5
L. fermenti, No. 1	2
L. fermenti, No. 2	1
L. salivarius	1
L. helveticus	1

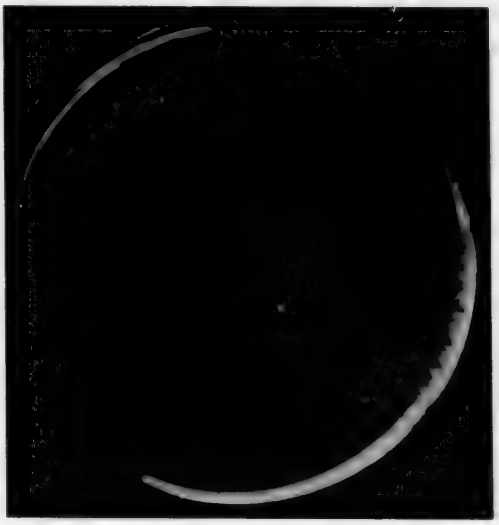

Fig. 1.—Plaques produced by Lactobacillus species NLF bacteriophage in 20 hours (agar-layer technic).

approximately 1 to 4 mm. in diameter (Fig. 1). Broth suspensions of the phage, prepared as described from these plaques, had titers of approximately 5×10^8 plaque-producing particles per milliliter.

Freeman[7] and Groman[8] reported the production of virulent lysogenic *Corynebacterium diphtheriae* from avirulent strains by exposure to bacteriophage. It is possible that a similar bacterium-bacteriophage relationship could develop among the bacteria of the oropharyngeal region. This might help to explain the sometimes sudden and inexplicable appearance of certain oral and pharyngeal diseases.

SUMMARY

Mouth wash specimens were tested for lytic action against 12 strains of lactobacillus. From a pooled mouth wash specimen, bacteriophage specific for a nonlactose-fermenting lactobacillus was isolated and stocked in broth. The possible use of lactobacilli bacteriophages in identification of lactobacilli is discussed. A possible relationship between oral bacteriophages and oropharyngeal infections is mentioned.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

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1. The Maximum Forces Exerted on the Dentition by the Perioral and Lingual Musculature. *William L. Kydd, School of Dentistry, University of Washington, Seattle, Washington.* Evaluation of forces exerted on the dentition by perioral and lingual musculature was measured by means of a plastic pressure device upon which the maximal forces of the tongue and lips were exerted. The plastic pressure device was coupled to a P-23 Stratham pressure transducer which constituted one leg of a Wheatstone Bridge. The signal was recorded on a Sanborn oscillograph. Maximal forces recorded were as follows: (1) tongue against upper anterior teeth, 8.05 lbs./sq. in.; (2) tongue against lower anterior teeth, 5.7 lbs./sq. in.; (3) tongue against either first and second molar areas, 1.3 lbs./sq. in.; (4) upper lip against alveolar process and maxillary anterior teeth, 4.4 lbs./sq. in.; and (5) lower lip against mandibular teeth and alveolar process, 4.1 lbs./sq. in.

2. Aspects of Growth in the Cranium, Mandible, and Teeth of the Rabbit as Revealed Through the Use of Alizarin and Metallic Implants. *L. C. Erickson, and A. Ogilvie, School of Dentistry, University of Washington, Seattle, Washington.* By tracing the movements of metallic implants with serial radiographs and observing the deposit of intraperitoneally injected alizarin red in 5 New Zealand rabbits, it was possible to determine the pattern of cranial growth and rate of growth at the frontal-parietal and frontal-nasal sutures, in the dentine, and the eruption of the anterior teeth. Findings were as follows: (1) the major portion of cranial growth took place at the suture lines and was 5 times as rapid at the frontal-nasal suture as at the frontal-parietal; (2) the cranium showed marked appositional growth on all internal and external surfaces; (3) the growth sites in the mandible were at the posterior border of the ascending ramus, in the condylar region, and in the alveolar process; (4) the average daily increment of the dential growth was 11.46 microns per day, and (5) the mean rate of anterior tooth eruption was 1.71 mm. per week.

3. Acid Formation in Sucrose by Salivary Action as a Means of Predicting New Caries Experience. *Marjorie K. Gardner, Marshall L. Snyder, and Donald R. Porter, The Dental School of the University of Oregon, Portland, Oregon.* Acid formation in sucrose broth by salivary activity as a means of estimating or predicting caries experience in the manner described by Rickles was tested in our project designed to evaluate laboratory tests for this purpose. Specimens of stimulated saliva were collected monthly from the study group

during the school year and submitted for lactobacillus count, Snyder Test, and salivary amylase, as well as acid formation in sucrose. Clinical examinations conducted with mouth mirror and explorer with supporting bite-wing radiographs were made at 6-month intervals. Our evaluation for predictability was limited, however, to a single set of saliva samples from 134 children and the data compared with the increment of new carious surfaces recorded 10 to 11 months later. Correlation studies indicated that acid formation in sucrose by salivary action could not be used to predict new caries experience in these children.

4. Certain Autonomic Responses of Children to a Specific Dental Stress. *Thompson M. Lewis and David B. Law, School of Dentistry, University of Washington, Seattle, Washington.* The psychophysical reactions of children undergoing a routine dental procedure could be accurately determined by a multichannel electroencephalograph adapted to receive and record on paper the following responses: (1) galvanic skin, (2) face temperature, (3) hand temperature, and (4) heart rate. Impulses from electrodes and thermisters, properly placed on a child in a dental chair, were transmitted by cable to the polygraph located in an isolated room. After careful standardization of action and words by the dentist, the reactions on 9 boys and 9 girls (5½ to 7 yr.) having similar dental experience histories were recorded during prophylaxis. Each patient rested 10 minutes and then was subjected to a 4-minute pumice prophylaxis without the mother present. This procedure was duplicated with the mother in the room. The entire experiment was repeated at a later date but reversed in respect to attendance of the mothers. There was no significant difference in the children's emotional response to prophylaxis whether mothers were or were not present.

5. A Qualitative Study of Stress Patterns in Various Cavity Preparations by Means of Photoelastic Analysis. *Richard Weigel and Jack Paris, The Dental School of the University of Oregon, Portland, Oregon.* Forces involved in mastication and stresses produced in teeth were investigated by photoelastic analysis using gelatin as the test material since it has been reported to be 600 times as photoelastically sensitive as Catalin. Satisfactory molds were made from rectangular wax blocks (2.0 by 0.5 by 0.5 in.) highly polished and invested with a soft mixture of plaster which was boiled out after setting. Various types of cavity restoration were carved from 3/8 inch plaster sheet. A plaster replica was glued topside down to a sheet of window glass and a prepared mold centered over the replica. The remaining space was filled with 13 per cent gelatin. This was kept at refrigeration temperature until ready for use, at which time the gelatin model with its plaster restoration was removed from the mold and its response to occlusal loads observed and photographed through a polariscope. Gelatin models could be used without appreciable deformation under occlusal loads up to 150 Gm. From a series of observations it was concluded that rounded line and point angles were superior to straight line and point angles in preventing the concentration of stress in a given area.

6. Vasopressor Effects of Topical Epinephrine in Certain Dental Procedures. *J. H. Gogerty, H. A. Strand, A. L. Ogilvie, and J. H. Dillie, School of Dentistry, University of Washington, Seattle, Washington.* Experiments were conducted on the absorption of epinephrine in situations typically encountered in dental practice. Randomly selected dogs were prepared for the standard assay of epinephrine. Dental procedures simulated as closely as possible those used in the dental office. Different concentrations of racemic epinephrine, with or without zinc sulfate and benzyl alcohol, were applied 2 minutes sequentially in each of 17 dogs: (1) intact mucous membrane, (2) area of gingival laceration, (3) two gingivectomy areas, and (4) an apicoectomy area. Under these conditions, topically applied epinephrine was absorbed from all situations except the intact mucous membrane. The degree of absorption was related to the extent of exposure of the vascular bed: that is, little systemic absorption was found in gingival laceration, more in full quadrant gingivectomy, and most during the apicoectomy procedure.

7. Studies on Micrococcus Lactilyticus. *W. Sims, Dept. of Bacteriology, University of Oregon Dental School.* In recent years, several investigators have suggested that since the organism, *M. lactilyticus,* is able to ferment lactate to the salts of weaker acids, it might be a protective factor in dental caries. To determine whether this organism occurred in greater numbers in the mouths of caries-inactive, as opposed to caries-active individuals, plate counts of the organism in both types of saliva were performed. The caries incidence in each group was established by annual clinical examinations, including bite-wing radiographs. Wax stimulated specimens of saliva were collected and, after suitable dilution, inoculated onto plates of a modified Douglas lactate agar and Rogosa SL agar. Although there was a marked difference in caries rate and numbers of lactobacilli between the groups, the mean counts of *M. lactilyticus* did not differ significantly. It was concluded that the organism, *M. lactilyticus,* did not occur in greater numbers in the mouths of caries-inactive individuals than in those of caries-active subjects.

8. An Index for the Longitudinal Assessment of Dental Caries Activity. *Donald R. Porter and John A. Dudman, Dental School of the University of Oregon and Reed College, Portland, Oregon.* An index is described which is based on all the possibilities per tooth surface between time *a* and time *b* with due consideration and weighting for surfaces presenting uncertain information, lost by exfoliation or gained by eruption.

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